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THE VIABILITY REACTION OF SOME INDUCED AND SPONTANEOUS MUTATIONS IN BARLEY

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INDUCED MUTATIONS AND AGRICULTURAL FITNESS.

IN 1947 the senior author published a summarizing paper on mutations in agricultural plants. Further data, especially with regard to yield conditions, were given by GUSTAFSSON and MAC KEY (1948). Since then several new facts have accumulated. They confirm the general conclusion of the publications mentioned, *viz.* that some mutants may equal or even surpass the mother strains with regard to production, irrespective of this concerns the generative or the vegetative phase. Such is the case in species like barley, wheat, oats, flax, and peas, all of which are self-fertilizing. In addition, the mutants often improve their mother strains in special properties like stiffness of straw, earliness or quality. By such characters the agricultural fitness may be increased even more.

Recent results, obtained by German authors, add themselves to the conclusions gained by the Swedish Seed Association. FREISLEBEN and LEIN (1942, 1943, 1944) as well as STUBBE and BANDLOW (1947) enumerate a great number of interesting mutation types in barley. In their textbook on the theory of plant-breeding, KUCKUCK and MUDRA (1950) give further examples. Some of their data, obtained from unpublished sources, may need corroboration.

Meanwhile, the first »roentgen variety» has been released into the market. A strain selected out of X-rayed Svalöf white mustard (*Sinapis alba*) produces a seed yield four per cent above its mother variety and increases the oil production per hectare by six per cent (ANDERSSON, 1950). The new variety is called »Primex white mustard». Since the species is cross-fertilizing to a high degree, there may be some discussion about the new variety having arisen by means of mutation. However, one and the same original X_1-plant, from which the new variety originated, also produced distinct mutations as to flower colour and pod shape. These mutations segregate independently of one another. Consequently, the X-rays induced some very complex happenings in the original seed.

Mere selection within the rather uniform mother variety has afforded no types more high-productive or rich in oil, whereas X-rays have given rise to several pedigrees with an increased oil-content. Thus there is a high probability that the increase in seed yield and oil content, too, is due to the complex mutation or a set of independent mutations induced by the X-rays. Similar complex happenings have been revealed and analysed in barley, wheat and sweet lupine.

The gathered facts indicate that also in self-fertilizing organisms new commercial varieties will ultimately be obtained by means of ionizing radiations. In a recent article FRANKEL (1949) has expressed the view that the positive mutants, described in barley, for instance with an improved stiffness of straw, might be due to deficiencies. This idea simulates the obsolete view that X-rays are merely destructive. The morphological mutant erectoides 1 and the physiological mutant 44/2 (»tall, late») both possess a superior stiffness of the straw (GUSTAFSSON, 1947). They were obtained in 1934—1935 and have been tested in yield trials since 1940. The *ten year* data conclusively prove that erectoides 1 is slightly superior in yield to Golden barley, its mother strain, and that the mutant 44/2 certainly varies considerably year after year but nevertheless surpasses Golden barley with three or four per cent. The idea, expressed by FRANKEL, that the gain in agricultural fitness is due to deficiencies seems to us like the idea that evolution is taking place by mere loss mutations. Only in cases when an organism is rich in duplicated loci, is polyploid or lives under extreme conditions, may deficiencies imply an evolutionary progress.

However, the primary aim of this paper is not to prove the possibilities of increasing variation and improving yield by artificial mutation. It is rather to examine the viability reaction and its relative issue. Under certain exterior conditions one and the same mutant may improve viability, under other conditions decrease it. In fact, also mutants, which are generally regarded as deleterious, show at times favourable properties which make them fit for special environments (BRÜCHER, 1943; GUSTAFSSON, 1947, p. 91). To some degree this depends on pleiotropic effects. However, the viability reaction itself may change entirely with definite changes of the environment. A recent survey of these problems is to be found in the work of SCHMALHAUSEN (1949).

The experiments, discussed in this paper, have been supported by grants from the Agricultural Research Council of Sweden.

THE YIELD PRODUCTION OF SOME VITAL MUTANTS IN BARLEY.

In this section seven mutants arisen out of Maja barley will be analysed with regard to yield production under different year conditions and after variable amounts of manuring. The mother strain was until a few years ago the most productive barley variety in Scandinavia. The mutants have taken part in parallel yield trials, carried out in the com-

TABLE 1. *Yield properties in seven mutants of Maja barley.*

Strain	Yield in kg per hectare 1945	1946	1947	1948	1944	1949	Σ	Average relative proportion
Maja barley	3990	4410	4900	5420	5930	6160	—	—
Erectoides 12	101,3	100,1	105,5	98,9	99,7	94,6	600,1	100,0
» 13	101,0	93,9	96,3	94,5	94,1	96,0	575,8	96,0
» 16	100,5	97,6	96,7	93,4	87,9	81,7	557,8	93,0
Bright-green 2	108,8	100,7	93,9	101,3	96,3	94,0	595,0	99,2
»Straw-stiff, early»; 44/18	106,5	99,3	108,2	92,6	94,1	94,3	595,0	99,2
»Seeds differently coloured»; 44/30 ...	102,8	105,0	102,9	97,4	102,8	89,9	600,8	100,1
»Late, broad-leafed»; 44/31	98,0	107,7	99,6	96,7	100,2	100,4	602,6	100,4
Σ	718,9	704,3	703,1	674,8	675,1	650,9	4127,1	98,3
Average relative proportion	102,7	100,6	100,4	96,4	96,4	93,0	98,3	—

mon manner by the Barley Department of the Swedish Seed Association at the Svalöf Station. They include four morphological types (erectoides 12, 13, 16 and bright-green 2), as well as three physiological types

TABLE 2. *Physiological properties in seven mutants of Maja barley.*

Strain	Strength of straw (1—10) 1944—1949	Ripening time (days) 1944—1949	Protein content (% dry matter) 1944—1948	1000-kernel weight (gr) 1944—1949	Hectolitre weight (kg) 1944—1949	Sieve fraction I; >2,8 mm., % 1944—1949
Maja barley	8,2	105,3	9,0	40,4	67,9	32,3
Erectoides 12 ..	+ 0,7	+ 0,5	— 0,1	— 0,3	+ 0,9	+ 3,5
» 13 ..	+ 1,6	— 0,5	+ 0,6	— 2,6	+ 0,8	— 2,1
» 16 ..	+ 1,5	— 5,3	+ 0,6	+ 1,0	— 2,4	— 1,7
Bright-green 2..	+ 0,7	+ 0,2	+ 0,1	— 0,3	+ 0,1	+ 1,0
44/18	+ 0,8	— 2,0	+ 0,4	+ 1,4	+ 1,5	+ 5,2
44/30	+ 0,8	— 0,2	+ 0,2	+ 0,0	+ 0,1	— 17,2
44/31	+ 0,6	+ 0,2	+ 0,2	— 2,3	— 1,0	— 7,6

Fig. 1. Mutant showing increased straw stiffness (July, 1948; 500 kg of saltpetre per hectare). Erectoides 13.

(44/18: »straw-stiff, early»; 44/30: »seeds differently coloured»; 44/31: »broad-leafed, late»). See GUSTAFSSON, 1947, pp. 60—64. The plot size was circa 30 m^2 and the plots were randomized in three repeats. The figures of Table 1 indicate that two of the morphological mutants equal the mother strain as to yield and that the same is the case with regard to all the physiological mutants.

Before turning to the main issue of this section, some further properties relating to the agricultural fitness of the mutants will be considered, *viz.* stiffness of straw, earliness, kernel and hectolitre weight, sifting results, and protein content. The six year data are shown in Table 2.

Immediately before harvest time the stiffness of the straw was yearly recorded by Mr. C. BERGSTRÖM, agronomist, after a scale of 1—10. All the selected mutants are

Fig. 2. The mother strain, Maja barley (July, 1948; 500 kg of saltpetre per hectare).

superior in this respect, especially the erectoides mutants 13 and 16 (Figs. 1—3). In fact, the former mutant appears to be one of the straw-stiffest varieties ever cultivated at Svalöf. With regard to earliness, erectoides 16 and 44/18 imply distinct changes of improvement. The 1000-kernel weight has increased in the same two mutants, the hectolitre weight, an important feature, in one of them, 44/18. The sifting results often give a valuable information with regard to seed size and seed shape. Four seed portions are normally obtained: (1) seeds remaining on the coarsest sieve, 2,8 mm., (2) seeds remaining on the middle-sized sieve, 2,5 mm., (3) seeds remaining on the smallest sieve, 2,2 mm., and (4) the scrap, usually discarded, passing through even this last-mentioned sieve. For brewing purposes well-developed, rounded seeds are desirable. The data indicate that erectoides 12 and 44/18 are superior to the mother strain. Very noticeable is the poor result in 44/30.

The protein content is distinctly raised in erectoides 13 and 16, probably also in 44/18.

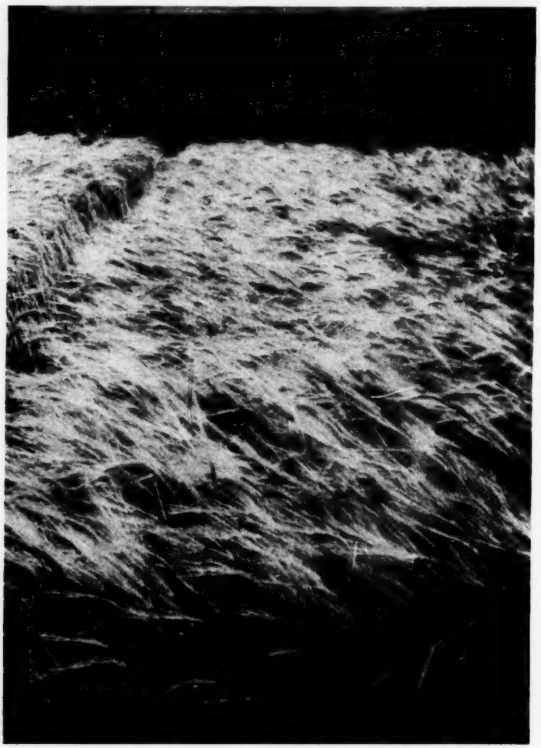

Fig. 3. Mutant showing increased straw stiffness (July, 1948; 500 kg of saltpetre per hectare). Erectoides 16.

The physiological mutant 44/18 implies a considerable improvement in several features. In addition, it equals the mother strain with regard to yield capacity.

Every farmer knows that cereal production is greatly influenced by various unpredictable conditions of the year, relating to time of sowing, moisture and temperature conditions in the germination and seedling stage, amount and distribution of precipitation during vegetation and ripening periods, summer heat, and harvest conditions. The considerable yearly fluctuation is reflected in the mother strain, too. In the experiments reported here Maja barley produced 3990 kg per hectare in 1945 against 6160 kg in 1949. Arranging the hectare crops in a rising scale, we obtain the following sequence of year production: 1945, 1946, 1947,

1948, 1944, 1949. The normal yield trials of the Barley Department give a similar order. The official trials give a rather similar picture for the southernmost province of Sweden, where Svalöf is situated. The agreement is in fact conspicuous:

The yearly production of Maja barley.

	1945	1946	1947	1948	1944	1949	
Mutation trials (Svalöf)	3990	4410	4900	5420	5930	6160	kg/hectare
Trials of the Barley Department (Svalöf)	3760	4590	4790	5420	5810	5590	»
Official trials (the province of Skåne)	3460	4540	3610	4610	4570	—	»

Hence, there is scarcely any doubt that the years 1945, 1946 and 1947 were characterized by conditions leading to a fairly poor production, in contrast to the years 1944, 1948 and 1949 which among South-Swedish farmers are ranked as especially favourable.

A close study of Table 1 reveals the fact that there is an opposite tendency in production capacity of mother strain and mutants. In years having a low average production (1945, 1946, 1947) the mutants are superior to Maja barley. In years with a high average production (1944, 1948, 1949) they are distinctly inferior. An analysis of variance presents the following results (v^2 = ratio of variance; BONNIER and TEDIN, 1940):

	Degrees of freedom	Sum of squares	Mean square
Between years	5	461,48	92,296
» mutants	6	308,09	51,348
Rest	30	526,94	17,565
Sum	41	1296,51	—

Ratios of variance: Between years, $v^2 = 5{,}255$, $P = 0{,}001$
» mutants, $v^2 = 2{,}923$, $0{,}05 > P > 0{,}01$

That this behaviour is no characteristic of these Maja mutants exclusively, is shown by some analysed vital mutants in Golden and Bonus barley.

	1945	1946	1947	1949	1942	1944	1943	1948	
Golden barley	3940	3950	4240	4640	4840	5020	5120	5280	kg/hectare
Mean of three mutants, including erectoides 1	106,4	107,9	106,0	102,0	103,4	105,2	101,3	101,0	
Bonus barley	3800	4500	5080	5810	—	—	—	6650	kg/hectare
Bright-green 3 (mean of two pedigrees)	105,9	103,0	96,2	95,9	—	—	—	90,1	

The mutants considered here, especially the well-studied ones out of Maja barley, are relatively seen less productive in favourable years than in unfavourable ones. This is in marked contrast to artificial polyploids, produced by autopolyploidy in several agricultural plants (LEVAN, 1945), the weak points of which are exposed under harsh environmental conditions. Evidently vital mutants and autopolyploids often react according to different viability schemes. The physiological unbalance seems to be greater in the autopolyploids.

Effect of manuring. — The yield trials of barley mutants are now laid out in such a manner that the reaction to high or low manurings are simultaneously studied. For this reason the experimental plots receive different amounts of nitrate of lime: 200, 300—400, 500 kg per hectare. In 1944—1947 200, 300 and 400 kg were given, in 1948—1949, 200, 400 and 500 kg. If the barley strains are not sufficiently stiff in straw, they react to high nitrogen manurings by a severe lodging. Hence the important feature of straw stiffness may be easily and correctly estimated by increased manurings.

In 1944 to 1949 the seven Maja mutants, recorded above, give the following yield results. In all four groups Maja barley is put as 100:

Manuring	200	300	400	500 kg/hectare
Relative yield of seven mutants	93,60	106,60	99,37	93,20
No. of experimental plots	42	28	42	14

There is for Maja barley itself, as well as for the mutants, a considerable rise in yield with the increase of manuring. In Maja barley the production rises according to the proportion 100 : 105 : 112 : 113 for the four nitrogen amounts recorded above. The data relating to the mutants indicate that these do not profit from very high manurings (500 kg per hectare) to the same degree as the mother strain. In plots with a low amount of manuring they are similarly inferior. By medium manurings, on the contrary, they profit more readily than Maja barley. An analysis of variance gives the following results:

	Degrees of freedom	Sum of squares	Mean square
Between years	5	1872,61	374,52
» mutants	6	970,09	161,68
» manurings	2	2523,54	1261,77
Primary interactions			
Year-mutant	30	1504,10	50,14
Year-manuring	10	1740,57	174,06
Mutant-manuring	12	370,51	30,88
Secondary interaction (= error)	60	3046,80	50,78
Sum	125	12028,22	—

Ratios of variance: Between years, $v^2 = 7{,}375$, $P < 0{,}001$
» mutants, $v^2 = 3{,}183$, $0{,}01 > P > 0{,}001$
» manurings, $v^2 = 24{,}840$, $P < 0{,}001$
Years-mutants, $v^2 = 1{,}987$, $P > 0{,}2$
Years-manurings, $v^2 = 3{,}427$, $P = 0{,}001$
Mutants-manurings, $v^2 = 0{,}608$, $P > 0{,}2$

$$\frac{\text{Manurings}}{\text{Years-manurings}},\ v^2 = 7{,}249,\ P = 0{,}001$$

$$\frac{\text{Manurings}}{\text{Mutants-manurings}},\ v^2 = 40{,}860,\ P < 0{,}001$$

By this analysis it has been proved, we think, that the viability reaction of the vital mutants studied here differs considerably from what is characteristic of their mother strain. The mutants are at their best in years with a minimum or medium crop production. Furthermore, in the experiments reported they gain by moderate amounts of manuring.

YIELD AND COMPETITIVE ABILITY.

The mutant to be considered here: bright-green 4, arose out of Bonus barley (01518), a sister strain to the well-known and high-productive Ymer barley. It differs from the mother strain by its distinct bright-green colour on stems, leaves and spikes. The peculiar colour is associated with a loss of waxiness. Bright-green mutations are by no means rare. In general they are characterized by an astonishingly rich production, seen in relation to their exterior character of »loss-mutations». Already in the twenties Dr. HANS TEDIN, then head of the Barley Department, found a spontaneous non-waxy mutant in progenies of Primus barley. It equalled the mother strain with regard to yield (GUSTAFSSON, 1947, p. 15). Four bright-green mutants, with a somewhat different distribution of the colour, lie on an average fairly close to their respective mother strains:

Bright-green 1,	1944—1946	— 91,3 %	(mother strain = 100)
» 2,	1944—1949	— 99,1 »	»
» 3,	1945—1949	— 96,4 »	»
» 4,	1946—1949	— 94,7 »	»
Average		— 95,4 %	

A fifth bright-green mutant differs in many respects from the other representatives of this kind and is, judged from its partial seed sterility, associated with chromosome disturbances of some kind. Hence it shows a conspicuous inferiority.

Crossings between the various bright-green mutants have so far produced no bright-green F_1's. Their striking phenotypical appearance depends consequently on different hereditary factors, although nothing regarding their number is known.

Crossings of bright-green 4 with its mother strain produce no decrease in fertility. Since such a decrease is a sensitive test of chromosome disturbances (translocations), no major chromosome rearrangements arose simultaneously with the factorial change causing the bright-green colour. Two sets of data are available, one of which is given below:

	Fertility	No. of plants	No. of flowers
Bonus barley	95,52 % ± 0,44 %	94	2166
Normal segregate	96,21 » ± 0,43 »	84	1929
Heterozygote	95,82 » ± 0,33 »	150	3538
Mutant	95,92 » ± 0,35 »	134	3158

The heterozygote segregates regularly according to a monofactorial scheme (1 : 2 : 1), as shown by the following analysis:

Year	Normal homozygote	Heterozygote	Mutant	Sum	χ^2	P
1946	20	39	25	84	1,023	0,70—0,50
1947	41	95	32	168	3,845	0,20—0,10
1948	55	98	43	196	1,470	0,50—0,30
Sum	116	232	100	448	1,714	0,50—0,30

Test for heterogeneity: $0{,}50 > P > 0{,}30$. The dominance of the waxiness is to all appearance complete.

The competitive ability of homozygous bright-green 4 and its mono-heterozygote with Bonus barley was tested in such a way that segregating progenies were continually broken up in the years 1945—1948. From every plant within a segregating progeny twenty kernels were sown with fifteen centimetres between the rows and five centimetres between the kernels in a row. The individual plants were not classified beforehand as to their genotypes, this in order not to risk the random distribution of the progenies. In the winter season the number of spikes, kernels and the kernel weight were determined for all the plants to be analysed. In every tenth or eleventh row twenty kernels of the mother strain (Bonus barley) were sown. The genotypical constitution was determined in the summer season one year after the harvesting. About one fourth of the rows consisted of either the normal or the mutant homozygote and the remaining two fourths of the heterozygote. The actual number was that presented above: 116 : 232 : 100.

Hence three sets of data may be compared: (1) the production of the mutants *in pure cultures* (yield trials of the common type), (2) the

production of normal, heterozygous and mutant progenies *in entire rows*, (3) the production of individual homozygous and heterozygous plants *within a row*. The highest degree of genotypical competition prevails of course within the row. But there is a conspicuous competition between the rows, too, both with regard to the root and the stem parts. Only in years when the climatic conditions prevent a full development of the stand, either through a poor germination or a low amount of tillering, the competition is largely annulled. Such was the case in 1947.

TABLE 3. *Yield conditions of bright-green 4.*

	1946	1947	1948	1949
Bonus barley	4500	5080	5810	6650 kg/hectare
Bright-green 4 Pedigree 1	94,1	93,4	97,6	99,8
» 2	—	83,7	97,9	96,3

Average mutant yield: (1) all data — $94{,}69 \pm 2{,}01$ %
» » » (2) except 1947, ped. 2 — $96{,}52 \pm 0{,}99$ %
Difference Bonus/1: $0{,}01 > P > 0{,}001$
Bonus/2: $P < 0{,}001$

The yield results are given in Table 3. If all the data are included, the production of bright-green 4 *in pure cultures* approximates 5 per cent less than Bonus barley itself. The actual figure is $94{,}69 \pm 2{,}01$ %, with a t-value for the difference of 2,642 and $0{,}01 > P > 0{,}001$. If the aberrant figure relating to pedigree 2 in 1947 is excluded, the average yield rises to $96{,}52 \pm 0{,}99$ %, $t = 3{,}515$, $P < 0{,}001$. Consequently in pure cultures, where competition with other genotypes is out of question, bright-green 4 is circa 5 per cent inferior to the mother strain.

Let us then turn to the competition *between different rows*. The figures of Table 4 show that the average kernel weight per row is higher in the mutated homozygote and the heterozygote than for the normal

TABLE 4. *Row competition of bright-green 4.*
(Relative kernel weights per row; 1946—1948.)

Material	1946	1947	1948	Average
Bonus barley	103,2	89,3	98,9	97,1
Normal segregate	100	100	100	100
Heterozygote	101,7	96,6	108,8	102,4
Mutant	102,3	87,9[1]	115,6	101,4
No. of rows	84	168	196	—
No. of plants/row	17,1	11,3	15,5	—

[1] *Cf.* Table 6: year 1947.

TABLE 5. *Row competition of bright-green 4 (1948).*

Material	No. of rows	No. of harvested plants	No. of plants per row	Kernel weight per row	Kernel weight per plant
Bonus barley	23	351	15,26 ± 0,36	55,52 ± 2,34	3,64 ± 0,15
Normal segregate	55	835	15,18 ± 0,34	54,89 ± 1,76	3,62 ± 0,12
Heterozygote	98	1612	16,45 ± 0,25	59,71 ± 1,31	3,63 ± 0,08
Mutant	43	657	15,28 ± 0,38	63,44 ± 2,93	4,15 ± 0,19

Differences:

No. of plants per row: Heterozygote/normal — $0{,}01 > P > 0{,}001$
Heterozygote/Bonus — $P = 0{,}01$
Heterozygote/mutant — $P = 0{,}01$

Kernel weight per row: Mutant/normal — $0{,}02 > P > 0{,}01$
Mutant/Bonus — $0{,}05 > P > 0{,}02$
Heterozygote/normal — $0{,}05 > P > 0{,}02$

Kernel weight per plant: Mutant/normal — $0{,}02 > P > 0{,}01$
Mutant/Bonus — $0{,}05 > P > 0{,}02$
Mutant/heterozygote — $0{,}02 > P > 0{,}01$

segregate and for Bonus barley. The mutant lies four per cent higher than Bonus barley and one per cent higher than the normal homozygote, extracted from segregating material. This difference must be seen in relation to its 5 per cent lower yield in pure cultures. The material of 1948 is large enough to allow a statistical evaluation. As shown by Table 5, the difference between bright-green 4 and Bonus barley gives $0{,}05 > P > 0{,}02$ for the yield, if calculated per row. The difference between mutant and normal homozygote gives a value of $0{,}02 > P > 0{,}01$. If calculated per plant the corresponding values are similar. Consequently we may infer that in 1948 bright-green 4 was decidedly superior to the normal homozygote as well as to Bonus barley itself. The figures of 1947 and 1946 are less clear. Neither of these two years render significant differences. This is so even in 1947, where the bright-green mutant appears to have suffered strikingly.

However, the low production in 1947 gets its explanation after considering the poor seed germination and the low average number of plants reaching maturity. This number is not higher than 11,3 plants per row, against 15,5 and 17,1 in 1946 and 1948. Consequently, there is no special competition present between plants and between rows. Putting the mean of kernel weights in Bonus barley and normal homozygotes as 100 the mutant will give a relative value of 93, *i. e.* exactly the same figure as pedigree 1 in pure cultures (93,4).

The data concerning plant competition *within the rows* indicate a

TABLE 6. *Plant competition in heterozygous rows.*
(Relative kernel weights per plant; 1945—1947.)

Material	1945	1946	1947	Average
Normal segregate	100	100	100	100
Heterozygote	103,5	97,1	104,2	101,6
Mutant	*110,2*	*104,6*	*94,1*	*103,0*
Plants per row	*18,0*	*15,8*	*11,4*	—
No. of plants	141	221	240	—

similar behaviour (Table 6). The average production of mutant : heterozygote : normal homozygote runs as 103 : 102 : 100. The mutant had its maximum in 1945 when the plant survival and in consequence the competition was at its top. Lowest was the production in 1947 with its poor germination and survival. The low production in 1947 is in fact due to the inhibited competition, not to any special climatic influences working on the full-grown plants. This interpretation is supported by the following analysis:

(A) *11 or less than 11 plants per row.*

	No. of spikes per plant	Prop.	No. of kernels per plant	Prop.	Kernel weight, gram, per plant	Prop.	No. of plants
Normal type	5,53	100	101,4	100	4,39	100	30
Heterozygote	6,07	110	108,3	107	4,67	106	61
Bright-green	5,29	*96*	92,1	*91*	3,87	*88*	35
(B) *12 or more than 12 plants per row.*							
Normal type	5,10	100	89,8	100	3,84	100	30
Heterozygote	5,51	108	109,7	122	4,25	111	63
Bright-green	5,24	*103*	98,2	*109*	4.29	*112*	21

This analysis clearly indicates a different behaviour of the mutant in thin and in dense stands. In rows with 11 or less than 11 plants there is a small inferiority of the vegetative parts (number of spikes per plant) and a distinct inferiority of the generative parts, most conspicuous with regard to kernel weight. In rows showing a pronounced competition (12 or more plants) the spike number is somewhat higher in the mutant than in the normal homozygote, the kernel number is decidedly higher and the kernel weight even more so. The mutant produces — relatively seen — less numerous and less heavy kernels when it grows freely than in dense stands. The contrast between normal type and mutant is indeed striking.

Therefore we may conclude that the mutant »bright-green 4», and probably its monoheterozygote, too, is inferior to the normal type and the mother strain in pure cultures, where no competition of different genotypes takes place, but that it gradually gains a distinct superiority when competition becomes intenser.

SUNESON and WIEBE (1942), as well as SUNESON (1949), have elucidated the competitive values of four commercial strains of barley: Atlas, Club Mariout, Hero and Vaughn. The experiment series lasted for sixteen years (1933—1948). The yield of the four strains proportionated 100 : 100 : 105 : 107 in pure stand. Vaughn and Hero were the highest-yielding varieties. After sixteen years, starting from a seed mixture containing equal amounts of seed, Atlas came to predominate (88 % of all plants), mixed with 11 % of Club Mariout and no more than 1 % of Hero and Vaughn together. Thus the two highest-yielding varieties show a very low degree of competitive ability.

An experimental series, carried out by ÅKERBERG (1940), is of interest in this connection. He found that the vegetative production capacity of two varieties of timothy may vary considerably with the density of the stand. One variety is superior in dense, another one in thin stands. ÅKERBERG worked with a cross-fertilizing species, characterized by a more or less conspicuous heterogeneity, and relates the difference in production capacity to the number of weak or poor segregates, which are of no influence under a high competition but will survive and diminish production in thin stands.

LETHAL GENES AND INCREASED VIABILITY.

In the late twenties NILSSON-EHLE discovered two mutants, spontaneously arisen out of Golden barley. One is a viridoalbina type according to the system of GUSTAFSSON (1940), the other a yellowish, slightly green type. They were originally denoted as »albina 7» and »xantha 3». These mutations act as recessive lethals killing the plants in the seedling stage. Their dihybrid was found to produce heterosis (GUSTAFSSON, 1946, 1947 b). Since they originated from one and the same strain, and one most homogeneous, and no differences have ever been found to distinguish the two monohybrids except for their mode of segregation, GUSTAFSSON concluded that under certain conditions and in certain cases lethal mutations may increase viability in the heterozygous state, although they unavoidably kill the organism when homozygous.

STERN (1948) produced a great number of X-chromosome lethals in *Drosophila melanogaster* and tested their effects on viability in heterozygous females. He found most of them to decrease viability. However, his results cannot be ranked as definite. Previous experiments, especially those carried out by MASING (1938, 1939), dealt with autosome material. Lethals in the first chromosome or in the second and third chromosomes may react differently owing to the continuous elimination of males in the case of X-chromosome lethals. In addition, long deletions, small deficiencies and point

mutations may produce entirely different results, depending on which genes or chromosome parts are involved.

The starting point for the set of experiments to be recorded here was however another: Do any environmental conditions exist, where lethal mutations will increase viability *also in the homozygous state*? Extreme chlorophyll mutations cannot as a rule assimilate. Consequently they die. Hence we must search for properties like germination ability, juvenile growth rate, seed resistance, *etc.*, in which the recessives may actually surpass the normal homozygotes. Especially suitable are treatments with extreme heat, which actually occur also in nature.

The monohybrids of »albina 7» and »xantha 3» give rise to three different genotypes in the proportion 1 : 2 : 1, one of which (the recessive) is lethal. The dihybrid gives rise to four viable genotypes in the proportion 1 : 2 : 2 : 4 and to five more genotypes, two of which imply xantha-individuals and three imply albinas. The genotypes are:

Monohybrid offspring	Dihybrid offspring
Albina 7	
AA — normal homozygote	AABB — normal homozygote
Aa — segregating 3 : 1	AABb — segregating 3 : 1
aa — dies	AaBB — » 3 : 1
	AaBb — » 9 : 3 : 4
Xantha 3	
BB — normal homozygote	AAbb — xantha, dies
Bb — segregating 3 : 1	Aabb — » »
bb — dies	aaBB — white, dies
	aaBb — » »
	aabb — » »

Segregation tests will immediately reveal the genotype of any individual plant in a monohybrid or dihybrid progeny. After such tests have been carried through, seeds from plants of identical genotypical constitution may be mixed together and kept under controlled conditions. Differences in seed size are easily avoided by passing the seed portions through sieves of different widths. Then seed material of one and the same sieve width is used. The experiments of this section always refer to such standardized seed materials.

Seeds of one and the same genotype may react to one and the same heat treatment in many ways owing to casual conditions. Low water content and small size are known to make seeds of cereal species resistant to extreme temperatures. In addition, protein constitution influences the resistance. In one year a seed material will be severely

damaged by a few hours' treatment with 80° C. In another year a temperature of 90° C given in like manner and to seemingly like material will be of no harm. Such was the case in 1947, the seed material of which was employed in the experiments to be reported here.

Identical seed portions (sieve widths 2,8, 2,5 or 2,2 mm) from monoheterozygous plant material (Aa and Bb) as well as their homozygous sister plants (AA and BB) were given two temperatures of circa 100° C (99 and 101°) for one, two and four hours. In the following survey the variation of time will not be considered, since it does not influence the results reported here. From other points of view it will be discussed in another paper.

The weaker treatment with circa 99° gives a seed germination in sand of $49{,}33 \pm 1{,}44$ % for the green homozygotes and $54{,}83 \pm 1{,}44$ % for the monohybrids. The difference corresponds to a t-value of 2,696 with $0{,}01 > P > 0{,}001$, *i. e.* an evident sign of a monoheterozygous superiority in resistance. With the higher temperature of 101° the normal homozygote germinates to $25{,}92 \pm 1{,}27$ % and the monohybrid offspring to $21{,}00 \pm 1{,}18$ %. Also here the difference is significant ($t = 2{,}844$, $0{,}01 > P > 0{,}001$). In this case the monoheterozygous offspring are somewhat inferior.

The figures run as follows:

	Segregation	Germination	P	No. of seeds
Control	603—182 = 785	98,13 %		800
99° Monohybrids	510—148 = 658	54,83 »	$0{,}01 > P > 0{,}001$	1200
99° Green homozygotes	—	49,33 »		1200
101° Monohybrids	205—47 = 252	21,00 »	$0{,}01 > P > 0{,}001$	1200
101° Green homozygotes	—	25,92 »		1200

The calculated viability of the different genotypes is at 99° for normal homozygotes 49 %, heterozygotes 59 % and recessives 53 %, at 101° for normal homozygotes 26 %, heterozygotes 20 % and recessives 17 %.

Offspring from dihybrid material, consisting of diheterozygotes, monoheterozygotes and green homozygotes, were given 90, 95, 100 and 104° for one, two and four hours. The most severe treatment with 104° caused an almost complete lethality. Of AABB and AABb no material germinated, of AaBB and AaBb seeds germinated to 0,5 %. Each of the four genotype groups comprised 300 seeds. On the other hand, a treatment with 90° did not markedly influence germination. The untreated control material germinated to 97 %. The green homozygotes (AABB) gave a figure of 96 %. The total offspring of monohybrid plants (AABb

and AaBB), including seedlings of green homozygotes, heterozygotes and recessives, germinated to 92 %. The total offspring of dihybrid plants (AaBb) gave a value of 95 %.

With 95°, however, the situation is entirely different. The data are found in the following survey:

	Germination	No. of seeds
(1) Untreated control material	98,00 ± 0,28 %	2400
(2) Offspring of green homozygotes (AABB)	53,50 ± 2,03 »	600
(3) » » monohybrid plants (AaBB or AABb)	80,42 ± 1,15 »	1200
(4) » » dihybrid plants (AaBb)	82,33 ± 1,56 »	600

Differences: $t_{3-2} = 11{,}55$; $P < 0{,}001$
$t_{4-2} = 11{,}26$; $P < 0{,}001$

Consequently there is a conspicuous dihybrid and monohybrid superiority.

With 100° the results are contrary. The germination is the following:

	Germination	No. of seeds
(1) Untreated control material	98,00 ± 0,28 %	2400
(2) Offspring of green homozygotes (AABB)	24,00 ± 1,75 »	600
(3) » » monohybrid plants (AABb or AaBB)	18,50 ± 1,12 »	1200
(4) » » dihybrid plants (AaBb)	21,83 ± 1,69 »	600

Differences: $t_{3-2} = 2{,}644$; $0{,}01 > P > 0{,}001$
$t_{4-2} = 0{,}892$; $P > 0{,}20$

There is evidence that the homozygotes are superior to the monohybrids, but not to the dihybrids. In like manner as done for the original monoheterozygotes, the relative viability of the dihybrid material can be determined. We find the following figures:

		95°	100°
	Normal homozygotes (AABB)	53,50 %	24,00 %
Derived monohybrid material	Monoheterozygotes (AABb and AaBB)	74,24 »	18,73 »
	Recessives (AAbb and aaBB)	76,41 »	17,25 »
Dihybrid material	Mono- and diheterozygotes (AABb, AaBB and AaBb)	82,93 »	23,88 »
	Recessives (AAbb, aaBB, Aabb, aaBb and aabb)	88,43 »	18,83 »

It is therefore evident that at a temperature range between 90 and 100°, when seed germination drastically decreases, there is a special stage when heterozygous and recessive seeds are superior to the normal homozygotes. Below and above this stage the superiority disappears or is transferred into its contrast.

A further case was recorded, showing that lethal recessives may surpass their normal dominants as well as their heterozygotes with respect to seedling growth. In one of the heat-treatments recorded above (90°) the leaf-lengths of every plant was measured at a definite time (eleven days) after the sowing in sand.

The data obtained indicate that the mutant seedlings grow faster and become taller than the normal homozygotes. Their absolute superiority amounts to 2,3 % ($t = 2{,}008$, $0{,}1 > P > 0{,}05$). The normals in their turn are superior to the mixed offspring of monohybrids and dihybrids. The difference is 4,9 % ($t = 1{,}96$, $0{,}1 > P > 0{,}05$). Both differences border to significance. Highly significant is the difference between mutant seedlings and the heterozygous mixture (7,04 %, $t = 8{,}12$, $P < 0{,}001$).

This interesting fact may be interpreted in various ways. We cannot, of course, generalize from this single although comprehensive set of experiments. We conclude, however, that under certain conditions some metabolic processes of fundamental importance, relating to growth conditions, can be influenced by lethalizing mutations in a positive direction.

The three cases analysed in this section show a variable behaviour of the normal homozygotes, the heterozygotes and the recessive mutants. The viability reaction *for seed production* is, according to GUSTAFSSON (1947): heterozygote > normal homozygote (> mutant); *for seed germination* at a definite temperature stage: mutant > heterozygote > normal homozygote, and with regard to *seedling growth* under certain exterior conditions: mutant > normal homozygote > heterozygote.

DISCUSSION.

Analysing agricultural species with regard to mutant viability, we reach the following conclusions.

Mutations may directly, without the help of numerous modifiers and small mutations, and very markedly, indeed, change the morphology and physiology of a species. The flower structure, the spikelet, the grain, the straw can be entirely rearranged (GUSTAFSSON, 1947; STUBBE and BANDLOW, 1947; MAC KEY, NYBOM, unpubl.). Striking examples of similar kind were described for *Antirrhinum* by STUBBE and WETTSTEIN. No less drastic are some mutations with regard to physiological properties like earliness or lateness (LUTHER SMITH; as well as GUSTAFSSON and MAC KEY, 1948), causing the time of maturity of summer cereals to vary with two months or more. Such is also the case with regard to height: giants and dwarfs of various kinds have been obtained; with

regard to kernel size: specially interesting is a barley mutant increasing kernel size by 50 per cent; with regard to leaf breadth: one mutant, called *phragmitoides*, shows almost reed-like leaves; and so on.

Many striking morphological and physiological mutants are remarkably good from a viability point of view. With this definite result as a back-ground we adhere to the view that evolutionary changes may take place in nature, too, by means of abrupt mutations, altering the morphological and physiological reactions by single steps, or, what appears to be more probable, by means of drastic changes combined with small mutations, each of which does not markedly influence appearance and behaviour. Drastic mutants do occur. Some are remarkably vital. This is the actual state of things.

The data gathered in this paper illustrate the complexity of the viability reaction. There are mutations that directly increase viability, even under conditions favourable to the mother variety. There are mutations that in pure cultures are less viable than their ancestor strain but surpass it in competition. There are mutations, which are specialized in their requirements and consequently improve yield under a narrow set of exterior conditions. Others are superior in an extreme type of environment. Mutations may be lethal or semilethal when homozygous but fully viable or »over-viable» in the heterozygous state. Nature is able to use and work with the entire range of fully viable to fully lethal mutations. Pleiotropic effects are so common and so diverse that scarcely any mutation can be denoted as always and entirely worthless.

The positive value of some negative mutations cannot be disregarded also in human affairs (GUSTAFSSON, 1947 b). This has been made clear, although not directly concluded, by RÜDIN in 1941. He says summarizingly (p. 128): »Wenn es sich nun also auch nachweisen lässt, dass in unserem unvereingenommenen gesammelten Material von Genialen und deren Verwandten die geistige Gesundheit in ganz einwandfreier Weise vorherrscht, so sind doch die zwei Erbstörungen manisch-depressives Irresein und Schizophrenie, mit Ausnahme gerade die Schizophrenie bei den genialen Probanden selbst, wo ja die Ziffer nur 1 % beträgt, also die hauptsächlichen endogenen Geistesstörungen gegenüber ihrer Häufigkeit bei der Durchschnittsbevölkerung und gegenüber Angehörigen einer sozial ähnlich oder gleich hoch geschichteten Bevölkerung deutlich erhöht. Diese Differenz ist, wenn sie auch bei Vermehrung des Materials nicht so gross zu bleiben braucht (!), als Differenz immerhin statistisch gesichert.»

This statement is in line with the results presented in this paper.

What is advantageous or deleterious, what is superior or inferior, what is sound and what is sick, must be seen in relation to the type of environment, to the interreaction of numerous genes and chromosomal changes and, not least, to the past happenings in the species or the population. Viability is a highly relative phenomenon.

Self-fertilizing species, where homozygous strains can be obtained and kept pure for long periods of time, afford a valuable material for the study of single mutations and their effects on viability. The mutated factor is stripped off all modifying influences from genes and rearrangements acting in the heterozygous condition. They do in no way complicate the viability reaction. The agricultural field, which often uses self-fertilizing species, implies a specialized type of ecological niche. However, nature itself is so rich in localities and niches of various kinds that some of them can be ranked as no less extreme than the agricultural field. Here the viability reaction can be tested in an adequate way by comparing seed, straw or tuber yields, depending on which part of the plant builds up the crop. The agricultural conditions permit a close control of the viability reaction.

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Communications from the Swedish Seed Association. No. 27.

THE PRODUCTION OF MUTATIONS BY CAFFEINE

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I. INTRODUCTION.

IN an earlier communication from this laboratory (FRIES and KIHLMAN, 1948), it was demonstrated that conidia of the fungus *Ophiostoma multiannulatum*, when treated with caffeine, to a certain extent gave rise to monoconidial mycelia with altered physiological characters. These deviating mycelia were by analogy named mutants, since they physiologically in all respects behaved like the so-called biochemical, or physiological mutants earlier obtained in this fungus after x-ray irradiation. All these types of mutants are characterized by their incapacity of utilizing the simple, »minimal» medium sufficient for the wild type, unless it is supplemented with some additional nutrient, or growth factor, different in the different types of mutants. Although always a great number of morphologically deviating mycelia were also observed after the treatment with caffeine, only the types of physiological mutations mentioned were isolated and studied, partly because they are more distinctly characterized than the morphological ones, and partly because a special isolation technique was elaborated for them.

As it seemed desirable to elucidate the real nature of the caffeine effect, the following study was undertaken. First and foremost it had to be established, at least in a few cases, whether the deviating monoconidial mycelia obtained after a treatment with caffeine were really mutants, *i. e.* if their specific characters were inheritable. This proving to be the case, the question remained, what sort of mechanism was responsible for the accumulation of mutants: a selective, or a mutagenic action of the caffeine.

The methods used in this study agree, unless otherwise stated, completely with those described in earlier publications (FRIES, 1948 b, 1949).

II. THE GENETIC CHARACTER OF THE PHYSIOLOGICALLY DEFICIENT STRAINS OBTAINED WITH CAFFEINE.

Since the physiologically deficient strains obtained after treatment with caffeine exhibited about the same general behaviour, degree of

stability, *etc.* in physiological experiments as the corresponding types of strains which had been obtained as mutations following x-ray irradiation, there seemed to be no doubt that the former were also real mutants. Five cases were, however, more thoroughly tested in this respect, *viz.* Nos. 2185 (hypoxanthine-less), 2186 (arginine-less), 2193 (parathiotrophic), 2194 (inositol-less), and 2213 (niacin-less). All of them represented the incompatibility group, +. Each one was combined with a —-mycelium, No. 6 (wild type), on agar containing 0,2 per cent malt extract. Ripe ascospores, produced in 4—6 days, were plated out on malt agar, and monosporous mycelia were isolated in as early a stage of development as possible. Some hundreds of these f_1-mycelia were physiologically tested. It turned out that, in all crosses, the deficiency of the »plus-parent» could also be found among some of the f_1-mycelia. In the cross 2185 × 6, for instance, there was a very regular segregation, 16 of the 32 monosporous mycelia being hypoxanthine-less (10 +, and 6 —), and the other 16 wild type (7 +, and 9 —).

Thus, the generalization seems to be allowed that the great majority, or perhaps all of the physiologically deficient strains obtained with caffeine, are mutants. In the following, therefore, these strains are called mutants throughout, whether the heritability of the specific character has been tested or not.

Furthermore, a few strains were examined as to their tendency to mutate back to wild type. Most of them seemed to be perfectly stable, and even after a long time no growth occurred in minimal medium. This does of course not necessarily imply that reverse mutations did not occur also in the apparently stable strains: they might be very rare, or perhaps prevented from further development under prevailing circumstances. Two strains, however, No. 2194 (inositol-less), and No. 2200 (parathiotrophic), produced indisputable reversals to wild type.

III. FLUCTUATION TESTS.

In searching for an explanation of the high percentage of physiological mutations among the conidia treated with caffeine, only two possibilities seem to be worthy of consideration, namely a selective or a mutagenic action of the treatment. In the first case, the caffeine would act more strongly killing upon normal conidia than upon mutated ones, thus gradually increasing the percentage of mutant conidia among the survivors. This action would be similar to that observed in starvation cultures of *Ophiostoma* (FRIES, 1948 a and b), where the absence of one

essential ingredient of the medium produced a selection in favour of physiologically deficient mutants. Fortunately, there exist some ways of distinguishing between such a selective and a true mutagenic effect by analysing the results of repeated experiments.

The first method here employed is similar to the so-called »fluctuation test», which has been used in studies of different sorts by various bacteriologists (*e. g.*, LEWIS, 1934; KRISTENSEN, 1940; DEMEREC, 1946; WITKIN, 1947), and is particularly worked out by LURIA and DELBRÜCK (1943; DELBRÜCK, 1945; LURIA, 1945; see also NEWCOMBE, 1949). The basic principles are equally valid in the case of fungal cultures consisting of conidia propagating by budding, although it must be borne in mind that this particular case of *Ophiostoma* is different from the above bacterial fluctuation tests, in so far as the nutritional mutants involved can be expected to respond differently than wild type conidia, even to a complete medium.

It is evident that even in a complete medium, where arising physiological mutants of *Ophiostoma* have a certain possibility of continuing growth, the percentage of such mutants is always very low, the highest value ever established being 0,06 % (or rather 1 mutant of 1597 isolated conidia). In all complete medium cultures tested, wild type conidia seem to have a selective advantage over mutant conidia. Because of this very low percentage, it is very difficult, by means of a direct plating out, to estimate how much this background percentage might vary from one culture to another, although one must assume that rather great fluctuations occur. On the other hand, we have some information about the *qualitative* fluctuations within this physiologically deviating part of the conidial population, this information being gained from studies of starvation cultures (FRIES, 1948 a and b). As could also be theoretically expected in that case, considerable differences were observed in the qualitative composition of the mutant populations of different cultures.

If the mutants obtained after treatment with caffeine were the result of a selection, the yield of mutants could consequently be expected to vary considerably from one experiment to another, quantitatively as well as qualitatively; if the mutants had been induced by the treatment, on the other hand, no such differences should appear. An experiment, No. 737, with caffeine treatment of conidia was therefore carried out in exactly the same way as No. 723 published earlier by FRIES and KIHLMAN (1948). Of the 4802 monoconidial mycelia isolated, 36 proved to be mutants, *i. e.* 0,75 %. This figure is not considerably lower

TABLE 1. *Mutants of Ophiostoma multiannulatum obtained after treating the conidia with 0,2 per cent of caffeine.*

M3 = »modified medium 3» = minimal medium (without asparagine and inositol).

Inoculation culture medium	Complete medium + inositol								Complete medium + arginine			M3		Total
Caffeine treatment medium	M3 + asparagine + inositol								M3 + arginine			M3		
Strain tested	11	11				11			11		5	11		
Experiment No.	722	723				737			742		754	763		
Treatment in days	5	5	6	7	8	4	6	8	4	7	8	7	9	
Factor required:														
Reduced sulphur	—	—	—	—	—	1	—	—	—	—	—	2	—	3
Arginine	1	1	3	—	—	1	3	3	—	1	2	2	—	17
Lysine	1	1	1	1	—	—	1	—	—	—	—	—	—	5
Methionine	—	1	—	—	—	—	1	2	—	2	—	1	—	7
Other amino-acids	—	1	—	1	—	—	—	—	1	—	—	1	—	4
Uracil	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hypoxanthine	—	3	1	1	1	1	—	1	—	—	1	3	2	14
Inositol	3	7	5	5	3	6	5	5	—	3	2	9	1	54
Niacin	—	4	3	—	2	—	4	1	—	1	1	3	1	20
Biotin	1	—	1	—	—	—	1	—	—	—	—	1	—	4
Other vitamins	—	—	—	—	—	—	—	—	—	1	1	—	1	3
Number of mutants	6	18	14	8	6	9	15	12	1	8	7	22	5	131
Conidia isolated	286	2264	1202	880	398	1350	1444	2008	828	1382	1494	1885	606	16027
% living conidia	—	0,29	0,08	0,04	0,03	0,80	0,13	0,09	0,85	0,03	0,16	0,11	0,02	—
% mutant conidia	2,1	0,8	1,1	0,9	1,5	0,7	1,0	0,6	0,1	0,6	0,5	1,2	0,8	(0,8)
		1,0				0,7			0,4			1,1		

than that obtained in the above experiment, *viz.* 1 %, and Table 1 shows further that the different types of mutants in both cases occur in similar proportions, the inositol-less ones constituting the dominating group. The arginine-less, hypoxanthine-less, and niacin-less mutants are also well represented in these two experiments. The small experiment, No. 722 (in fact the very first performed with the caffeine treatment), also fits fairly well into this scheme. The surprisingly high percentage of mutants obtained in this experiment, *viz.* 2,1 %, should, however, not be ascribed too great a significance on account of the small number of mycelia isolated.

Although this, very limited, fluctuation test did not support the idea of a selective action of caffeine, I thought it worth while to scrutinize the two alternatives in further experiments of a somewhat different sort. In the three experiments now discussed, the conidia were subjected to the action of caffeine in a minimum nutrient solution (»modified No. 3») with an addition of inositol and asparagine, an arrangement that was merely »historically» conditioned. Since inositol was also added to the complete medium of the inoculation culture, the possibility seemed plausible that a selection was in operation, after all, and the high percentage of inositol-less mutants was the consequence of the fact that this type of mutant was relatively favoured as early as in the inoculation cultures.

Three experiments with caffeine were, therefore, performed, in which inositol (and asparagine) was omitted as an extra addition to the medium. In two of these experiments (Nos. 742 and 754), arginine, 10 mg per tube, was substituted for inositol. Despite this arrangement, inositol-less mutants represented the dominant type also in these experiments, as may be seen from Table 1. In one experiment (No. 763), finally, the medium used for the inoculation culture as well as for the caffeine treatment consisted of the ordinary minimum medium, without any further additions at all. In this experiment, where a great number of mutants were obtained, the composition of the mutant yield agreed completely with those of the earlier experiments, inositol-less still being the predominant type.

The last experiment is of a particular interest in judging the possibility of a selective action of caffeine. In the earlier experiments the inoculation cultures were grown in complete medium, thus permitting a multiplication of spontaneously arisen physiological mutations. In this last-mentioned experiment, however, the minimal medium was employed for the inoculation culture, where such mutations consequently had a

very slight chance to multiply. (A limited, »syntrophic» growth might occur.) It seems justified to assume, therefore, that the percentage of pre-existing mutant conidia in the material to be treated with caffeine must be much lower in this case than in the earlier experiments where a complete medium had been used for the inoculation cultures. If the effect of caffeine was selective, and not mutagenic, the yield of mutants in this last experiment would have been considerably inferior to those of the earlier ones.

Summarizing the results of these fluctuation tests, we may conclude that they definitely support the assumption of a mutagenic action of caffeine.

If the collections of mutants obtained by random isolation after treatment with caffeine or x-ray irradiation (as published, *e. g.*, in FRIES, 1947) are compared, some differences appear which call for some attention. Although almost the same types of mutations occur in both cases, some of them are much more common in one case than in the other. Thus, it looks as if parathiotrophic, lysine-less, uracil-less, and hypoxanthine-less mutants occur more frequently after x-ray irradiation than after caffeine-treatment, the reverse, however, being true for inositol-less and niacin-less mutants (see Table 3).

Since no striking specific effects have been met with in the experiments with chemical mutagens so far published (*cf.* HOROWITZ *et al.*, 1946; TATUM *et al.*, 1950), it seemed *a priori* more likely that some factor in the isolation procedure was responsible for the differences mentioned. A change in the composition of the complete medium, in order to improve it, had been made in 1948, and consequently this modification could be suspected in the first place to be the cause of the differences in the composition of the mutant yields.

In order to clarify this point, I made some experiments, *viz.* Nos. 783, 787, and 789, using the same complete medium as in the caffeine experiments, but with irradiation as the means of inducing mutations. Since the x-ray apparatus formerly employed was not available any longer, a Hanau Original quartz lamp was used for the irradiation. The conidia were exposed to ultraviolet irradiation until c. 95 per cent of them had been killed. After the treatment, the conidia were plated out in the complete agar medium, and the developing monoconidial mycelia were isolated in the ordinary way (FRIES, 1948 b).

Table 2 shows the results of these experiments. It appears that, contrary to earlier experiences with x-ray irradiated material, but in accordance with the caffeine-experiments, the inositol-less mutants re-

TABLE 2. *Mutants of Ophiostoma multiannulatum obtained after ultraviolet irradiation of conidia.*

Factor required	Number of mutants obtained in the different experiments			Total
	Expt. No. 783	Expt. No. 787	Expt. No. 789	
Reduced sulphur	2	1	1	4
Arginine	3	—	1	4
Lysine	1	—	2	3
Methionine	1	4	3	8
Other amino-acids	1	—	—	1
Uracil	2	—	1	3
Hypoxanthine	2	1	1	4
Inositol	8	3	5	16
Niacin	—	—	—	—
Biotin	—	—	—	—
Other vitamins	3	—	1	4
Number of mutants	23	9	15	47
Conidia isolated	2518	1249	2302	6069
% living conidia	1,9	6,3	6,3	—
% mutant conidia	0,91	0,72	0,65	(0,77)

presented the decidedly dominating type. They amounted to 34 per cent of all mutants, which corresponds to 41 per cent in the experiments with caffeine, and 7 per cent in those with x-ray irradiation.

While a difference in the effects of x-rays and of caffeine is at least conceivable, a similar difference in the effects of x-ray und ultraviolet irradiation can scarcely be assumed, in view of the great number of investigations on the mutagenic action of irradiation, where no differences have been found in the relative susceptibilities of different genes to these two sorts of irrradiation (*cf.* BEADLE and TATUM, 1945). Thus, the different results have probably not been caused by the different treatments of the conidia.

The most reasonable explanation seems to be found in the differences, already mentioned, of the composition of the complete medium. For unknown reasons the changed complete medium, which in addition to the earlier ingredients, malt and yeast extract, also contained hydrolyzed casein (0,25 %) and synthetic nutrient solution (»modified medium 3»), seems to favour the development of the inositol-less mycelia, or — which is more unprobable — to disfavour the other types.

As to the differences in the relative occurrence of some other types of mutations, the figures are too small, at least in the UV-series, to permit

TABLE 3. *A comparison between the mutant yields in Ophiostoma after different sorts of treatment.*

Factor required	Number of mutants obtained by			Percentage of mutants obtained by		
	x-rays	UV	caffeine	x-rays	UV	caffeine
Reduced sulphur	34	4	3	13,9	8,5	2,3
Arginine	42	4	17	17,2	8,5	13,0
Lysine	32	3	5	13,1	6,4	3,8
Methionine	9	8	7	3,7	17,0	5,3
Other amino-acids	6	1	4	2,4	2,1	3,1
Uracil	26	3	—	10,6	6,4	—
Hypoxanthine	51	4	14	20,8	8,5	10,6
Other purines	4	—	—	1,6	—	—
Inositol	18	16	54	7,4	34,1	41,2
Niacin	6	—	20	2,4	—	15,3
Biotin	2	—	4	0,8	—	3,1
Other vitamins	6	4	3	2,4	8,5	2,3
Not identified	9	—	—	3,7	—	—
Total	245	47	131	100,0	100,0	100,0

any conclusions as to any further substrate or treatment effects. Two cases, however, perhaps deserve attention, *viz.* the uracil-less and the niacin-less types. The former type of mutation is comparatively well represented in both of the irradiation series (11 % and 6 %, respectively), but no single uracil-less mutant had so far been isolated after a treatment with caffeine. The niacin-less type, on the other hand, occurs very rarely in the irradiated material, but is next to inositol-less the most common type among the caffeine-induced mutants. This might indicate that a certain specificity in the action of caffeine exists, compared with the two other, physical, induction methods, but the evidence is admittedly very weak.

IV. TESTS FOR DIFFERENTIAL KILLING.

1. THE EFFECT OF A BORDERLINE CONCENTRATION OF CAFFEINE ON THE CONIDIAL GROWTH.

If the assumption is true that the mutant conidia are more resistant to the killing effect of caffeine than normal conidia, one could also expect that the former would not be inhibited in their growth as much as the latter by lower — not killing, but growth-inhibiting — concentrations of caffeine. Experiments showed that the conidial growth of *Ophiostoma* was inhibited by a caffeine concentration between 0,150 and

TABLE 4. *The effect of four different concentrations of caffeine on the conidial growth of different strains of Ophiostoma.*

Strain No.	Nutritional character	Age of inoculation culture in days	Number of days until definite growth occurred			
			0,150 % caff.	0,175 % caff.	0,200 % caff.	0,225 % caff.
I b	Wild type	3	18	+	—	—
I b	» »	4	+	+	+	—
3	» »	2	6	20	+	+
3	» »	3	9	+	—	—
4	» »	3	8	+	—	—
4	» »	4	7	+	+	+
5	» »	2	5	+	+	+
5	» »	3	8	+	—	--
6	» »	2	10	+	+	+
6	» »	3	+	+	—	—
1989	Hypoxanthine-less	4	—	—	—	—
1990	Lysine-less	3	—	—	—	—
2001	Inositol-less	3	—	—	—	—
2070	Methionine-less	3	—	—	—	—
2076	Inositol-less	3	+	—	—	—
2116	» »	4	6	+	+	+
2121	Hypoxanthine-less	4	—	—	—	—
2128	Niacin-less	3	+	+	—	—
2144	Hypoxanthine-less	4	—	—	—	—
2148	Inositol-less	4	11	+	—	—
2154	Arginine-less	4	—	—	—	—
2165	Inositol-less	4	—	—	—	—
2167	Methionine-less	4	—	—	—	—
2187	Niacin-less	4	—	—	—	—
2193	Parathiotrophic	4	—	—	—	—
2203	Inositol-less	4	—	—	—	—
2211	Arginine-less	4	—	—	—	—

+ = a soon discontinued increase was observed in the turbidity of the conidial suspension.

0,175 per cent. A number of wild type and mutant strains were then tested as to their capacity of growing in shaken tubes with the ordinary synthetic nutrient medium supplemented with 0,150 or 0,175 per cent of caffeine. In the tubes which should be inoculated with mutant conidia, the nutrient solution also contained a sufficient amount of the substance specifically required by the mutant in question. The tubes were inoculated with well washed conidia from shaken-tube cultures grown 2 to 4 days in a complete medium. The inoculum contained in all cases c. 10 million conidia.

A caffeine concentration of 0,175 per cent inhibited growth completely in all tubes except one, No. 3 (wild type). The lower concentration, on the other hand, permitted a conidial growth in several cases, as may be seen from Table 4. Ordinarily, a visible increase in the turbidity of the cultures would have been observed as early as one or eventually two days after inoculation followed by a rapid growth until maximal optical density was reached. The presence of caffeine, however, caused a retardation, more or less pronounced in different cases. The wild type seemed on the whole to overcome the caffeine inhibition more easily than the mutant conidia. Of the ten tubes inoculated with wild type, only two did not reveal any definite growth after 20 days, when the experiment was discontinued. The mutant conidia, on the other hand, proved to be capable of further development only in two of the 17 tubes inoculated.

The result of this experiment seems to indicate that the mutant conidia, rather than the normal ones, are inhibited by caffeine in their development. This is of interest in connection with the fact that the treatment with caffeine in the mutation experiments lasts for a comparatively long time (more than 5 days). During this treatment a certain growth occurs, causing a slight increase of the turbidity of the conidial suspension. This growth is, however, soon finished, when each conidium has produced one or a few daughter conidia, or a short hypha. From the above described experiment with a caffeine concentration lower than that of the mutation experiments, it appears very probable that the spontaneously arisen mutant conidia, eventually present in the suspension under treatment, do not take part in this limited growth in any appreciable degree. This *could* mean — contrary to the above assumption forming the premise of this experiment — that the mutant conidia survive simply because they are more sensitive to the growth-inhibiting action of caffeine, a not quite inconceivable possibility in view of recent experiences as to the effect of penicillin on genetically heterogeneous populations of *Escherichia coli* (DAVIS, 1948, 1949; LEDERBERG and ZINDER, 1948).

The question of a selective effect, thus still left open, is more thoroughly treated in the following experiments.

2. THE KILLING EFFECT OF 0,2 PER CENT OF CAFFEINE ON PHYSIOLOGICALLY DIFFERENT STRAINS.

The experiment discussed above showed the capacity of different types of conidia to overcome the *growth-inhibiting* effect of sublethal

TABLE 5. *The killing effect of 0,2 per cent caffeine on various wild type and mutant strains of Ophiostoma.*

Strain No.	Nutritional character	Per cent living conidia after 4 days	6 days	8 days
3	Wild type	1,2	0,08	0,016
4	» »	1,0	0,08	0,015
5	» »	0,8	0,09	0,018
6	» »	1,0	0,09	0,016
11	» »	0,8	0,11	0,019
2001	Inositol-less	0,8	0,17	0,027
2076	» »	1,1	0,06	0,004
2165	» »	1,3	0,04	0,002
2121	Hypoxanthine-less	10,0	4,22	2,33
2128	Niacin-less	3,8	2,10	0,60

concentrations of caffeine. In another experiment, now to be described, the *killing* effect of a higher caffeine-concentration was studied. This concentration, 0,2 %, is always lethal, but the possibility existed that the death-rate is different for wild type and mutant conidia. The experiment was performed in the following way.

Washed conidia of ten different strains were tested, *viz.* Nos. 3, 4, 5, 6, 11 (all wild type), 2001, 2076, 2165 (all inositol-less), 2121 (hypoxanthine-less), and 2128 (niacin-less). The mutant strains were shortly before produced by caffeine-treatment. Conidia for inoculation were obtained in the usual way from complete medium in shaken cultures, washed three times, and then inoculated to the caffeine tubes, ten million conidia per tube. These tubes contained each 10 ml of the synthetic minimal medium plus 0,2 per cent caffeine, and after inoculation they were placed in the shaker apparatus at + 25° C. After 4, 6, and 8 days, samples of 1 ml were taken from the suspensions, diluted, and plated in complete agar medium for determining the percentage of surviving conidia in each tube.

The result is tabulated in Table 5. In the tubes with wild type conidia, the percentage of survivors decreased very uniformly (approximately according to an exponential formula), c. 1 % being alive after 4 days, c. 0,09 % after 6 days, and c. 0,017 % after 8 days. The inositol-less conidia behaved in about the same way: No. 2001 died somewhat more slowly, Nos. 2076 and 2165 somewhat faster. The two remaining mutants, on the other hand, were obviously much less sensitive to caffeine, the death-rate being much lower than that of the other strains tested. When the percentage of surviving conidia in the series with wild type and inositol-less conidia after 8 days caffeine-treatment had de-

creased to about 0,02 %, or lower, the two strains, No. 2121 (hypoxanthine-less) and No. 2128 (niacin-less), still exhibited a percentage of 2,3 % and 0,6 %, respectively, of living conidia.

Although the number of strains tested is low, the conclusion seems warranted that the resistance to caffeine varies between wider limits in the mutant strains than in the normal ones.

3. RECONSTRUCTION EXPERIMENTS.

If caffeine acts in a different way upon at least some mutant strains than upon the ordinary wild type strains, this would also appear from so-called »reconstruction experiments». This sort of experiment is analogous to the »test for differential survival» used by WITKIN (1947), and is earlier described in connection with my studies of starvation cultures (FRIES, 1948 a, p. 345). A more detailed description of the method seems therefore needless.

Four reconstruction experiments were performed, the results of which are summarized in Table 6. In each experiment a number of

TABLE 6. *Reconstruction experiments. The viability of two sorts of conidia in nutrient solution with 0,2 per cent caffeine.*

Treatment in days	Number of living conidia per ml (and %) Altogether		Wild type		Mutant		Living mutant conidia in % of all living	Living conidia: the ratio $\frac{\text{mutant}}{\text{wild type}}$
	(a) *No. 5 (wild type) + No. 2185 (hypoxanthine-less)*							
0	1200000	(100,0)	1104000	(100,0)	96000	(100,0)	8,0	0,087
5	12800	(1,1)	11648	(1,1)	1152	(1,2)	9,0	0,100
7	5120	(0,43)	4741	(0,43)	379	(0,39)	7,4	0,080
	(b) *No. 5 (wild type) + No. 2186 (arginine-less)*							
0	1300000	(100,0)	390000	(100,0)	910000	(100,0)	70	2,333
5	83200	(6,4)	4990	(1,3)	78210	(8,6)	94	15,667
7	15360	(1,2)	2150	(0,55)	13210	(1,45)	86	6,143
	(c) *No. 5 (wild type) + No. 2187 (niacin-less)*							
0	1120000	(100,0)	616000	(100,0)	504000	(100,0)	45	0,818
5	—	—	—	—	—	—	—	—
7	17600	(1,6)	3696	(0,60)	13904	(2,8)	79	3,762
	(d) *No. 5 (wild type) + No. 2191 (inositol-less)*							
0	1100000	(100,0)	1084600	(100,0)	15400	(100,0)	1,4	0,014
5	11680	(1,1)	11470	(1,1)	210	(1,8)	1,8	0,018
7	3744	(0,34)	3680	(0,34)	64	(0,42)	1,7	0,017

conidia from strain No. 5 (wild type) were mixed with a number of conidia from a certain mutant strain, the two combined strains being then run on complete agar medium and finally in shaken tubes with complete medium. The mixture of conidia obtained from each of these shaken cultures was transferred to a tube containing 10 ml of synthetic minimum nutrient solution to which 0,2 % caffeine had been added. C. 10 million conidia of the mixture were inoculated to each tube. After 5 and 7 days in the shaker at + 25° C samples of 1 ml were taken from each tube, and the number of living conidia of the two mixed sorts was determined.

As can be seen in Table 6, two of the mutant strains were less sensitive to the killing action of caffeine than the wild type strain, No. 5. The latter was obviously somewhat more resistant in these experiments than in earlier tests (see Table 5). The difference in sensitivity between No. 5 and the mutant strains was, however, not considerable, even in the most extreme cases. The greatest difference was attained after 5 days in the tube with No. 5 + No. 2186, where only 1,3 % of the wild type conidia were alive, but 8,6 % of the mutant conidia. In the two strains, Nos. 2185 and 2191, the death-rate was almost exactly the same as the average rate of the wild type strains.

From these experiments as well as from the other test for differential killing described above (Table 5), it appears that at least some mutant strains possess a higher degree of resistance to the killing action of caffeine than wild type strains. Whether this difference in response is high enough to account for the effect of caffeine in the mutation experiments, will be discussed in the following chapter.

V. DISCUSSION.

Since practically all sorts of physiological mutations known in *Ophiostoma* have been obtained by treating the conidia with caffeine, it may *a priori* appear highly improbable that the effect of this chemical could be explained as selective rather than mutagenic. When, later on, it was shown that caffeine causes structural changes in the chromosomes of *Allium* (KIHLMAN and LEVAN, 1949), and is thus capable of inducing mutations in this plant (at least of the type »chromosome mutation»), this indirectly supported the interpretation of caffeine being a mutagen in the case of *Ophiostoma*, too. The increase in the rate of mutations to phage-resistance in *Escherichia coli* observed by WITKIN

(quoted from DEMEREC, 1949) after a treatment with caffeine, also speaks in favour of this interpretation.

On the other hand, there were some other facts which entitled a more thorough analysis of the caffeine effect upon *Ophiostoma*. In the so-called starvation cultures a selective process had been found that acted in favour of all sorts of physiological mutations (FRIES, 1948 a and b). The existence of chemicals acting in the same way had earlier been discussed as a mere possibility (FRIES, 1948 b, p. 339), but was recently shown to be a reality in the case of penicillin (DAVIS, 1948, 1949; LEDERBERG and ZINDER, 1948). Finally it is evident that the duration of the treatment with caffeine in the mutation experiments is comparatively very long, and the risk for the development of selective processes is therefore probably greater than in short-time experiments with, for instance, nitrogen mustard. A shorter treatment with a higher concentration of caffeine does not seem to produce so many mutants. The possibility must, however, be taken into consideration that certain chemicals actually need a comparatively long time and a low concentration to act as mutagens.

The series of experiments where conidia of *Ophiostoma* had been treated with 0,2 per cent caffeine, can be regarded as a fluctuation test analogous to those earlier performed in the studies of starvation cultures. In the case of caffeine, however, no significant differences could be found between the single experiments as to the composition of their yields of mutants. Thus, from this point of view, nothing spoke in favour of the selection hypothesis. When a comparison was made with the results of UV-irradiation experiments, no striking differences in the above-mentioned respect could be detected either, as far as the limited material permitted any conclusions. The tests for differential killing and the reconstruction experiments presented, however, some facts which might indicate that a certain selection can also play a rôle in the production of mutations by caffeine. Independent of the strain employed, the wild type conidia possessed in each experiment the same sensitivity to the killing action of caffeine. The mutant strains differed more in this respect in so far as lower as well as higher degrees of sensitivity than that of the wild type strains were found. To make it possible to decide whether these differences would result in a selective enrichment of mutant conidia great enough to account for the whole increase in percentage of mutants after treatment with caffeine, the following calculation was made. It was assumed that one conidium of the mutant type in question was present for each 10000 wild type conidia

TABLE 7. *The percentage of mutant conidia after different periods of time in a caffeine-treated population assumed to contain originally 0,01 per cent of the mutant conidia in question.*

The percentage of all living conidia is in the first 5 cases (average values from Table 5): after 4 days 1,0 %, after 6 days 0,09 %, and after 8 days 0,017 %; in the last 4 cases (average values from Table 6): after 5 days 1,2 %, and after 7 days 0,48 %.

Calculated from Table	Strain No.	Physiological character	Per cent mutant conidia of all living conidia after days				
			4	5	6	7	8
5	2001	Inositol-less	0,008	—	0,019	—	0,016
	2076	» »	0,011	—	0,007	—	0,002
	2165	» »	0,014	—	0,004	—	0,001
	2121	Hypoxanthine-less	0,105	—	0,469	—	1,370
	2128	Niacin-less	0,039	—	0,233	—	0,353
6	2185	Hypoxanthine-less	—	0,011	—	0,009	—
	2186	Arginine-less	—	0,067	—	0,026	—
	2187	Niacin-less	—	—	—	0,047	—
	2191	Inositol-less	—	0,013	—	0,012	—

in the suspension to be treated with caffeine, this ratio being approximately equal to the average percentage of pre-existing mutant conidia (of a variety of types) in an untreated conidial suspension. On the assumption of a differential killing, according to the figures of Tables 5 and 6, the percentage of mutant conidia after different periods of time was calculated in each case. As may be seen in Table 7, the hypoxanthine-less strain No. 2121 was the only one possessing a sensitivity to caffeine low enough to give a final percentage value of c. 1 %, which is of the same order of magnitude as the values obtained in the mutation experiments. In all the other mutant strains the differential survival process did not result in any similar increase in percentage.

Thus, the possibility cannot be excluded that selection due to a different sensitivity to the killing effect of caffeine may play a rôle in some cases. To explain the results of the mutation experiments exclusively, or even to a large extent in this way, is, however, not possible, since that would require the assumption of each mutant conidium possessing a resistance to caffeine much higher than that actually found in the mutants studied, except in one single case. This state of things, together with the results of the fluctuation test, thus necessitates the acceptance of the other alternative proposed: that the caffeine effect is, at least mainly, due to a mutagenic activity of this chemical.

SUMMARY.

Monoconidial mutant mycelia, reversible as well as apparently irreversible ones, can be isolated from a conidial suspension of *Ophiostoma multiannulatum* after a treatment with caffeine. The chief aim of the present study was to elucidate whether this caffeine effect is due to selection, or to mutagenesis.

Irrespective of the medium used for the inoculation culture, no significant qualitative or quantitative differences could be found between the mutant yields of the various caffeine-treatment experiments, nor were these yields different from those of UV-irradiation experiments. These facts can be understood only by assuming a mutagenic activity of caffeine.

On the other hand, in a few cases mutant conidia exhibited a higher (as well as, in other cases, a lower) resistance to the killing action of caffeine compared with wild type conidia. A certain selection in favour of pre-existing mutant conidia may therefore in single instances operate in the presence of caffeine. However, this mechanism cannot, ordinarily, be responsible for more than an insignificant part of the caffeine effect.

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FERTILITY CONDITIONS OF TETRAPLOID RED CLOVER

I. SEED SETTING OF TETRAPLOID RED CLOVER IN THE PRESENCE OF HAPLOID POLLEN

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WORK has been in progress for the last few years at the Herbage Plant Department of the Swedish Seed Association with a view to elucidating some of the problems connected with the fertility conditions of the tetraploid red clover. In recent years the breeding of tetraploid clover has given very promising results (see, *e. g.*, LEVAN, 1948; FRANDSEN, 1948). In respect of vegetative development certain families have proved superior to the diploid varieties from which they were produced, and it is probable that in course of time they will be employed for practical cultivation. Unfortunately it is found in red clover, as in many other kinds of plants, that the tetraploids have a constantly lower seed setting ability than the diploids, a fact that was pointed out by both the aforementioned authors.

The cause of this low fertility in the tetraploids has not been clarified. Probably many different factors contribute to it. However, it is necessary to solve these problems and to produce types having a good seed setting if the artificially produced tetraploids are to gain any spread in practice.

If tetraploid varieties are to be marketed, it will be of great interest to know the extent to which the yield from a seed culture of a tetraploid variety is influenced by the presence of diploid plants. Firstly, it can be apprehended that fertilization of the tetraploids with haploid pollen would result in the production of triploids with the consequence that after a few generations the variety would come to consist of a mixture of plants having different chromosome numbers; secondly, it is conceivable that the haploid pollen would have a depressing effect on the seed yield on account of the triploid embryos aborting or giving rise to weak seeds.

MÜNTZING (1948) found that when tetraploid rye was grown close to diploids of the same species the seed production of the tetraploid was

noticeably much lower than when it was grown in isolation. He considered this reduced fertility to be due to the occurrence of spontaneous crosses between the diploids and tetraploids and the abortion of the triploid embryos then formed. OLSSON and RUFELT (1948) found an analogous condition in white mustard.

With a view to discovering whether similar conditions prevail in red clover there was started at Svalöf a series of investigations, of which the present paper is a first report.

In an experiment that must be regarded mainly as exploratory red clover plants were planted in the autumn of 1947 in small plots. One plot was entirely planted with the diploid Merkur, another only with tetraploid St. 586, while a third contained both diploid Merkur and tetraploids of St. 586. The plants developed quite satisfactorily the following spring, and just before flowering time insect-proof cages were placed over each of the plots. As soon as flowering began two humble-bees were introduced into each cage in order to perform the pollination. During the whole flowering season it was seen that two working bees were constantly present in each cage. On seed maturation each plot was harvested separately, and from the plot where tetraploids and diploids had been planted together each type of plants was harvested separately. Threshing was effected head by head, and the number of seeds per head was counted. The following results were obtained:

	In separate cages		In same cages	
	Diploid	Tetraploid	Diploid	Tetraploid
Number of heads	193	200	205	191
» » seeds/head	17,6 ± 1,47	12,2 ± 1,05	23,3 ± 1,69	7,0 ± 0,99

Grown in separate cages the diploids thus gave a better seed yield than the tetraploids, which agrees with earlier experiences of the fertility conditions in tetraploid red clover. The difference between the mean values is 5,4 ± 1,81 seeds per head, which is a statistically significant difference. When grown together in a common cage the two forms of clover showed a greater difference, *viz.* 16,3 ± 1,96. In this case the seed setting of the diploid was greater than that of the diploid grown in its special cage. However, probably this is wholly a consequence of the uncertainty of the experimental method employed. Against the background of this good seed setting the low seed production of the tetraploid in the same cage is especially striking. The difference between the tetraploids in the two cases is 5,2 ± 1,44, which is fully significant. Here, the poor seed setting of the tetraploid may be caused by disturbances due

to the presence of haploid pollen, but it may also be a consequence of the humble-bees having preferred the diploid because it possibly had a shorter corolla tube. It has in fact in earlier experiments at Svalöf been found that when they have free choice humble-bees prefer flowers with shorter tubes (JULÉN and ÅKERBERG, 1948).

In order to study the effect of the haploid pollen on the seed production of the tetraploids under better controlled conditions experiments were made with hand pollinations. Early in the spring of 1948 plants from the red clover trials sown the preceding spring were taken up and planted in pots. Two diploid varieties were used: Merkur and Wambåsa. Of the tetraploids the three varieties St. 586 (out of Merkur), St. 553 (out of Merkur × Wambåsa) and St. 588 (out of Offer) were chosen. At the time for flowering the pots with the well developed plants were put in an insect-proof crossing house, in which they were allowed to remain during the entire flowering season. Three kinds of crosses were made. Tetraploid flowers were pollinated partly with pollen obtained from other tetraploid plants, partly with pollen from different diploids, and finally also with a mixture of both kinds of pollen. The pollinations were carried out without previous emasculation. On each head the number of pollinated flowers was counted and was found to average 75,8. After the seed had matured and had been harvested, each head was threshed separately and the number of seeds per head was counted. Thereupon the percentage of seed production for each head was computed. The frequency distribution of this percentage over the three crossing groups was as follows.

Cross	Numb. of heads	Number of heads with average seed setting of 0-5 %	5-10 %	10-20 %	20-30 %	30-40 %	40-50 %	50 %	M%
Tetr. × Dipl.	38	15	7	5	1				5,9 ± 0,95
Tetr. × Tetr.	42	4	5	13	9	6	3	2	21,3 ± 2,28
Tetr. × (Tetr. + Dipl.)	15	11	3	1					3,9 ± 1,25

The highest mean percentage of seed formation, 21,3 ± 2,28, was shown by those crosses which had been carried out with diploid pollen. The diploid pollen gave a 15,4 percentage units higher set of seed than the haploid pollen alone, and 17,4 per cent more seed than when a mixture of diploid and haploid pollen was used. Both these differences are statistically significant. The difference of 2 percentage units between the crosses with haploid pollen and those with mixed pollen is not significant. Hence the haploid pollen has an equally disturbing effect when it occurs mixed with diploid pollen as when it is applied alone to

the pistil. It is of interest to note that of the 139 seeds obtained from the cross carried out with haploid pollen 62 or 44,6 per cent were shrivelled or undeveloped, whereas in the case of the cross with diploid pollen 87 or 13,9 per cent of the 625 seeds obtained could be classified as shrivelled.

The seeds obtained from the crosses with diploid pollen and from those with mixed pollen were set out to germinate. Before germination each cross was divided into three groups according to whether the seeds were well-filled, shrivelled or undeveloped. On the seedlings obtained root-tip fixations were made for chromosome-number determinations. When the chromosome number could not be determined on these roots, the seedlings in question were put into pots and later on fresh root-tip

TABLE 1. *Number of seeds obtained, of germinable seeds and of chromosome determinations.*

Type of cross	Seeds developed	Number of seeds	Number of germinated seeds	Number of chrom. No. determ.	Number of plants with the chromosome No. 28	21 ±
Tetr. × Dipl.	Well-filled	77	66	50	49	1
	Shrivelled	27	5	4	3	1
	Undeveloped	35	0	0	0	0
	Total	139	71	54	52	2
Tetr. × (Tetr. + Dipl.)	Well-filled	20	20	14	14	0
	Shrivelled	11	0	0	0	0
	Undeveloped	7	0	0	0	0
	Total	38	20	14	14	0

fixations were made on the plants that had developed normally. Some of the plants were weak and died before the radical system had developed sufficiently for root-tips to be obtainable. The number of seeds obtained, number of germinable seeds and number of chromosome determinations that could be made can be seen from Table 1.

It was chiefly the well-filled seed that germinated. After the cross with haploid pollen 5 germinable seeds were obtained in the group »shrivelled». One of these gave rise to a triploid plant, the rest of the chromosome-counted individuals were tetraploid. Of the 50 plants that had developed out of well-filled seed and whose chromosome number were counted, only one was a triploid while the others were tetraploid. No triploids could be found in the cross with mixed pollen.

It therefore seems as though presence of haploid pollen at the

fertilization of tetraploid red clover has in some way a disturbing action on the seed setting. On various occasions MÜNTZING has advanced the hypothesis that a corresponding phenomenon in rye depends on haploid pollen-tubes having a quicker rate of growth than diploid ones and thereby reaching and fertilizing the egg-cell sooner. The triploid embryos formed in the process are thought to abort. If such conditions can be demonstrated for red clover, this could account for the results emerging from the previously described experiments.

The following method was adopted to study the pollen-tube growth. From the previously mentioned tetraploid plants in the crossing house a number of flower-heads were selected, half of them being pollinated with haploid and the other half with diploid pollen. The flowers were not emasculated. For pollen-donors use was made of several different plants from each stock. There were, however, no self-fertilizations. The heads were snipped off and put in water indoor.

Staining of the pollen tubes was performed by the method using cotton blue described by DARLINGTON and LA COUR (1942). A solution of 0,08—1 % of cotton blue is dissolved in a solution of equal parts of lactid acid, phenol, glycerine and water. A dye concentration of 0,1 % was found suitable for the red clover material. At varying intervals of time after fertilization pistils were prepared and placed in a drop of dye solution on a microscope slide. The slide was placed for heating in a temperature of about 60° C for 1—1 $^1/_2$ hours, whereupon the pistil was crushed under a cover-glass.

The style of a tetraploid red clover pistil is about 12 mm long. Its upper third part is a solid structure and can only be crushed with difficulty. In this portion of the style it is therefore difficult to see the pollen tubes. The remainder of the style consists of a loose tissue, in which the pollen tubes stand out as dark-blue bands in the light-blue stylar tissue. During the crushing process this loose tissue is broken up and the pollen tubes have a tendency to tear themselves out of the stylar tissue or to be broken off, and this sometimes renders it difficult to determine their exact length.

It may be of interest to point out here that as a rule it could be established that one or a few pollen tubes had got ahead of the great majority. This is in agreement with results obtained by SILOW (1931). In red clover there is only one egg-cell to be fertilized in each pistil, and therefore the rate of growth of the longest pollen tube is, in this connection, of greater interest than the average rate. Therefore only the longest was measured. At the same time the length of the style was also

measured. The measurements were performed with an ocular micrometer at a magnification of 280 times.

Preliminary examinations showed that as a rule haploid pollen tubes had grown through the style in about four hours, and the fixation was therefore done about 2, 3 and 4 hours after the pollination. In all, 85 pistils with haploid tubes and 69 with diploid tubes gave good slides which could be analysed. The pollen tubes in 18 pistils of the former kind managed to grow through the whole style. In these cases it could not be decided how long a time had passed from pollination until the first pollen tube had reached the ovary. Hence the measurements obtained from these 18 pistils could not be used for a computation of the rate of pollen-tube growth.

No difference could be discovered in the average pistil length between the different strains; nor was it possible to establish any connection between the average rate of pollen-tube growth and the pistil length. In the further analysis of the material no account has therefore been taken of the pistil length.

A study of the growth rate of the pollen tubes gave the following values expressed in μ per minute:

Pollen from	Variation µ	Mean µ
Merkur (dipl.)	42—82	63,0
Wambåsa (dipl.)	44—84	58,8
St. 586 (tetr.)	26—50	35,0
» 553 (»)	22—60	37,4
» 588 (»)	42—58	35,2

The variation within each strain has accordingly been relatively considerable. However, no difference can be found between tubes from the different diploid varieties, nor between those of tetraploids.

On the other hand, a noticeable difference is observed between haploid and diploid pollen tubes. The former had a growth rate averaging $60{,}4 \pm 1{,}16$ μ/minute, while the corresponding value for the diploid tubes was as low as $36{,}1 \pm 1{,}00$. The difference between these values, $24{,}3 \pm 4{,}82$, gives a t value of $5{,}050$ and is accordingly fully significant.

Fig. 1 shows the measured lengths of the pollen tubes after different periods of time and the regression lines for the relation between time and length of haploid and diploid tubes, respectively. Provided the regression is linear the relation for the haploid tubes follows the equation $y = 4242{,}0 + 33{,}2\,x$, where y is the length of the pollen tubes and x is the

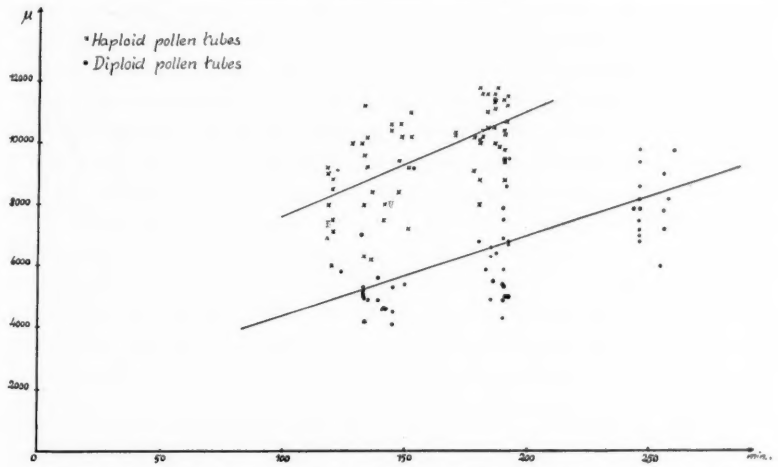

Fig. 1. Relationship between time after pollination and length of pollen tubes.

time expressed in minutes. For the diploid tubes the regression equation will be $y = 1806{,}5 + 25{,}9\,x$.

These regression lines do not run to the origo, and presumably they should in reality be curved. From Fig. 1, however, it is seen that curvature would probably be relatively slight within the interval in question. The figure also shows that the pollen tubes must have grown quicker during the first two hours after pollination than later, and that the initial growth rate of the haploid tubes had been substantially higher than that of the diploid tubes. The higher regression coefficient for haploid pollen tubes obtained in the investigation shows a tendency to a slightly higher growth rate of these tubes also between two and three hours. Great variation in the material makes, however, that no significant differences in this respect could be discovered.

The investigation disclosed that the haploid tubes had grown through the style in three to four hours. On the other hand, in no case could diploid tubes be found that had grown down after four hours, and the figure also shows that they require a substantially longer period of time in which to reach the embryo-sac. It is conceivable that this retardation may be a contributive cause of the generally low fertility of the tetraploid red clover. An investigation into this problem is in progress.

A study of Table 1 indicates that the well-filled seeds obtained from

a cross with haploid pollen were tetraploid in 52 of the 54 cases in which the chromosome number could be determined. As the flowers were not emasculated, it can be assumed that these seeds had been formed after selfing. This would presuppose a considerably higher self-fertility in the tetraploids than what is usual in diploid red clover. Such a self-fertility in tetraploids of species which are self-sterile in a diploid state has been demonstrated by, amongst others, LAWRENCE (1930), LEWIS (1943) and ATWOOD (1944). Most of the fertilizations have probably been carried out by haploid pollen grains, but the triploid embryos may have aborted at an early stage (the percentage of seed production was very low) or may have given rise to undeveloped and shrivelled seeds (the percentage of shrivelled seed was remarkably high). There is also a possibility that some of the plants which were so weak that they died before the chromosome number could be counted, were triploids.

In the progeny from the cross with mixed pollen no triploid could be demonstrated in any instance. But, as it can be established that the haploid tubes grow so much faster than the diploid tubes, it can be assumed that when a tetraploid red-clover flower is simultaneously pollinated with haploid and diploid pollen the great majority of fertilizations will be performed by haploid pollen tubes, with triploid embryos as a result. As in the case of fertilization with solely diploid pollen, these triploid embryos must be assumed to have aborted or possibly to have given rise to shrivelled and undeveloped seeds.

In embryological investigations carried out with *Lycopersicum pimpinellifolium*, COOPER and BRINK (1945) have shown that embryos arising after reciprocal crosses between diploids and tetraploids develop very slowly and are stifled after a time by the surrounding fast-growing somatic tissues. In the ripe fruit there are found many shrivelled seeds. MÜNTZING (1943) and OLSSON and RUFELT (1948) have also shown that aborting embryos and shrivelled seeds have been formed after crosses between tetraploids and diploids. As an explanation of the powerful disturbance in the seed production of tetraploid red clover that was found in the previously described experiments one may therefore venture to suggest that triploid embryos were formed but that these aborted or gave rise to non-vital seeds or plants.

For the practical seed cultivation of red clover a spontaneous crossing with diploids will doubtless be of small importance. If diploids and tetraploids are grown in adjoining fields there may admittedly be reason to apprehend a reduction in the seed yield, but usually the seed cultivations lie comparatively well isolated. From the standpoint of varietal

purity, on the other hand, any spontaneous crossing that may occur does not involve a serious risk, as the production of perfectly sound triploid seeds is very small. Moreover, the few triploid plants that are obtained are often very weak and soon succumb, and it is doubtless to be expected that any persisting triploid plants will have a very low fertility. These different factors cooperate to make spontaneous crossing from diploids in tetraploid red clover a matter of practically no importance.

SUMMARY.

When tetraploid red clover is pollinated with pollen of diploids the seed setting will be considerably lower than when pollination takes place with pollen from tetraploids. This has been ascertained by means of pollinations with the aid of humble-bees in cages as well as by hand pollinations. From hand pollination it could be established that the seed production was as weak when a mixture of haploid and diploid pollen was applied to the pistil as when haploid pollen alone was employed. In both cases a high percentage of shrivelled and undeveloped seeds resulted, most of which were not germinable. In the progeny from a cross with haploid pollen it was possible to determine the chromosome number in 54 cases. Only 2 of these 54 individuals were triploids, the rest were tetraploids. After crosses with mixed pollen all the individuals whose chromosome number could be fixed were tetraploids.

The growth rate of diploid pollen tubes in tetraploid tissue was determined as $36{,}1 \pm 1{,}00$ μ/min., that of haploid tubes under the same conditions was $60{,}4 \pm 1{,}16$ μ/min.

The majority of the fertilizations will therefore be carried out by haploid pollen grains, giving in consequence triploid embryos. These can be assumed to abort at an early stage or to give rise to poor and undeveloped seeds. The presence of pollen from diploid red clover therefore has a highly disturbing effect on the seed formation of the tetraploids.

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Communications from the Swedish Seed Association. No. 25.

A CLINICAL AND GENETICAL STUDY OF DISTURBED SKELETAL GROWTH (CHONDROHYPOPLASIA)

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INTRODUCTION.

SEVERAL inherited abnormalities of the human skeleton have been described. It is possible to refer these abnormalities to entirely different groups showing a different histological and clinical picture. We may consider the chondrodystrophic (or as some writers prefer to call them, achondroplastic) disturbances of skeletal growth as such a group. This is in agreement with the point of view of BAUER and BODE (1940). Within this group several different genotypical entities have been described. The theory of a genotypical entity is set forth by inference and may be based on a thorough analysis of *inter-* and intrafamilial variability only. There is a good reason to look upon the different types of brachydactyly and brachyphalangy as different genotypical entities, as they show a rather constant clinical picture within families but often a wide variation between families.

I agree with those who consider brachydactylism and chondrodystrophy genetically and pathologically related, but this by no means need or does imply that those two conditions form a genetic unity. They seem to be the effect of entirely different genes which are qualitatively much alike but differ quantitatively and topically. By chondrodystrophic disturbances is meant a group of inherited deviations from normality which are characterized pathologically by a defective development of the chondrous skeleton. The pathogenesis is not completely understood. It is, however, evident that vitamin deficiencies or endocrine disturbances have nothing to do with this condition. Above all the unilateral appearance of chondrodystrophy in some cases is a proof of this view (*cf.* NATHANSON, 1912).

The literature on chondrodystrophy has recently been reviewed by MØRCH (1941) in his study of chondrodystrophic dwarfs in Denmark. There will be no need for further consideration of that particular point.

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Considering inherited skeletal abnormalities, we are in an advantageous position so far as most of the genes in action here seem to have a very high penetrance. This is of great importance not only because it facilitates the genetical analysis but because the genetic variability and the mode of gene action found in these conditions may be a guide to a better understanding of other genetically determined diseases or defects in which conditions are much more complex, *i. e.* mental diseases.

There are some trends in modern medical genetics in which the value of statistics on large material is overestimated. Neglect to pay proper attention to the genotypical entity of the material will most often create confusion when calculations of the mode of inheritance are made. The selfevident fact that clinical and genotypical entities may and must often lack a close biological correlation is often forgotten. It is most important to examine the genotypical background of every clinical entity or syndrome to which we want to apply genetical and statistical analysis. As an example of this way of proceeding the work of SJÖGREN (1943) on hereditary ataxia should be mentioned. It was possible to separate rather conclusively two genotypical entities, one recessive and correlated to the syndrome known as Friedreich's ataxia and one dominant chiefly with cerebellar signs and symptoms, roughly corresponding to the type of Pierre Marie.

Furthermore I am convinced that the examination of large pedigrees, if only the propositus is selected, is a valuable complement when analysing the correlation between clinical and genotypical entities, especially so when considering dominant characters or more generally characters with a heterozygous manifestation. Many traits now considered exclusively recessive may have some slight heterozygous manifestation, *e.g.* physical, mental or biochemical. A diagnosis of such conductors would naturally have a great practical importance from the eugenic point of view. Here again the analysis of pedigrees may add some information.

Our experience from experimental genetics tells us that a recessive gene is not to be compared to something non-existing. When a careful analysis is carried out, some effect, even if it is very slight, will most often be found. This fact is worth a thorough consideration in medical genetics. That this is not a Utopia is shown by some recent observations which will be cited here. ANDREASSEN (1943), on examining 31 heterozygotes of hemophilia, found in 30 cases definite changes in the coagulation time. In colour blindness the normal gene in combination with the »protanope» or »deuteranope» gene gives normal eyes when studied

by STILLING's test-card. However, if these women are determined by an analysis of luminosity curves their heterozygosity may be revealed (DE VRIES, 1948).

Dominance or recessivity has for long been a matter of conjecture. It is better to ask: what is the difference between heterozygous and homozygous manifestation?

Returning to the scope of the present paper, I had the oppurtunity to study a relatively large family the propositus of which had been diagnosed as chondrodystrophy in an orthopædic clinic. This family was registered in our department during an investigation with a view to studying the effect of cousin marriages in a circumscribed area in North Sweden. Every marriage between first cousins was registered and investigated. Chondrodystrophy was found in no other family. A special interest arose when it was found that both parents had a small stature and showed signs of a slightly impaired skeletal growth.

The problem set forth to solve was whether the diagnosis of the propositus was correct from the clinical point of view and whether his condition was in some way genetically correlated to the slight disturbances found in his parents.

According to MØRCH (1941), who has presented a rather conclusive material, all cases of condrodystrophy are of a genotypical nature and the trait is transmitted as a dominant character, *i. e.* with heterozygous manifestation. It seemed that the present case might be an exception to this rule.

FAMILY RECORD.

The family was examined in June, 1947 and December, 1948. The research work in the Church Register has been facilitated by the interest and kind assistance of the Rev. H. DEGERMAN of Korpilombolc.

Most members of this family live in T-järvi, a small village in North Sweden not far from the Finnish border. Practically all the men are lumbermen. The women have to take care of the household and often of a small farm besides. Families with ten or more children are common. The life there is hard and primitive and the infant mortality is the highest in Sweden. Communications are still scanty and the nearest hospital is 300 kms away, though the distance to the district physician is only about 80 kms.

The observations took place during field work and have the character of clinical examinations without the aid of special equipment. It was not possible to add X-ray studies. However, the propositus and one

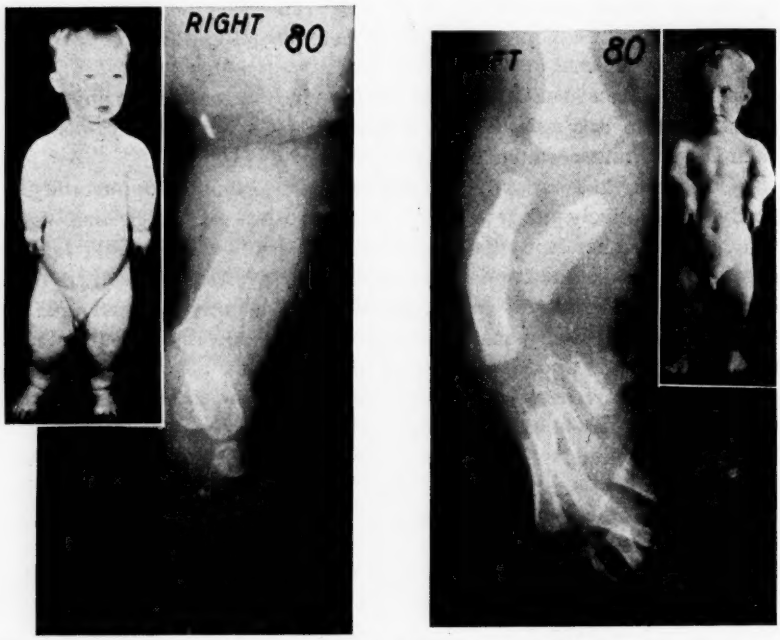


Fig. 1. *Propositus* showing gross malformations of chondrodystrophic type as an expression of homozygosity for the dominant »chondrohypoplastic» gene.

member of the family had visited the Orthopædic Clinic in Härnösand, and by courtesy of the chief physician, Dr. N. LINDSTRÖM, I could study the hospital records and radiograms.

The pedigree contains 123 persons (including the twin pair and the triplets), of whom it has been possible to examine 66. Before the examination there had been 28 deaths, and hence of all individuals living about 70 % have been examined.

The number of every person refers to the pedigree.

PROPOSITUS' FAMILY.

Propositus. J. A. E., born $^{19}/_{3}$, 1943, male. His mother was healthy during pregnancy. Delivery was normal in a breech presentation. It was observed at once that the child had exceptionally short arms and legs. He was breast fed for six months and received the usual treatment with cod liver oil.

When six months old the boy was taken to the Orthopædic Clinic in Härnösand (file No. 22 374). Both arms were very short. The forearms showed marked ulnar deviation. His legs, too, were very short but no deformity was observed. There was

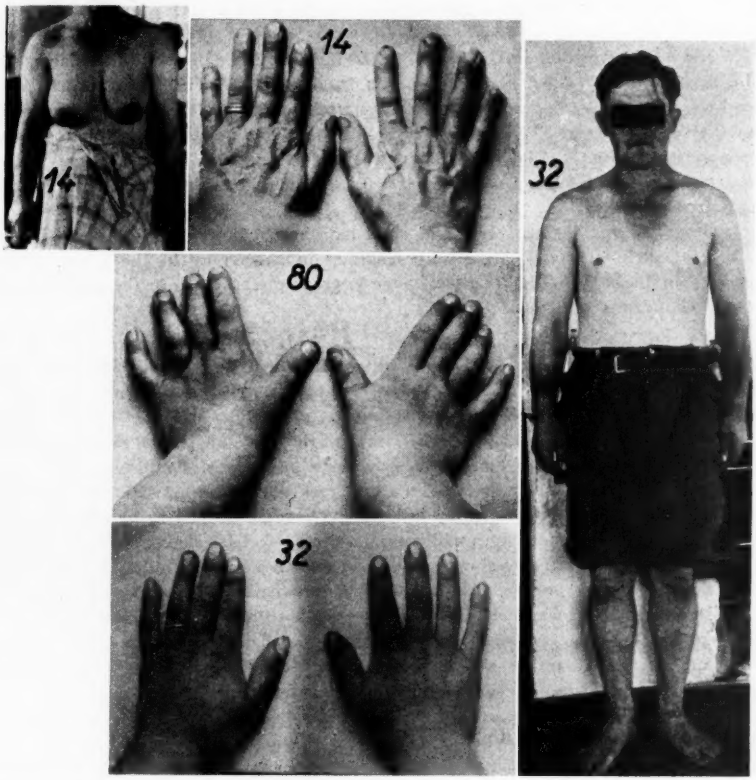


Fig. 2. The parents of the *propositus*, both showing slight impairment of skeletal growth diagnosed as chondrohypoplasia. Note body type, shortness of extremities and the slight brachydactylous hand type.

a defective extension of both knee joints. Otherwise the mobility of the joints was good. The head seemed enlarged. The fontanelles were tense.

X-ray examination ($^{20}/_{9}$, 1943): Both femora and humeri were short and clumsy. The fibulæ were only half the length of the tibiæ. The bones of the forearms were short and thick. The radii were curved with a radial convexity. *Diagnosis*: Congenital chondrodystrophy. Some of the radiograms are shown in Fig. 1.

The patient was re-examined at home on June 20, 1947 (*cf.* Fig. 1.). His arms and legs had grown a little the last few years and the shortness was less prominent but still remarkable. In other respects he had developed normally. He could walk at the age of one year and talked at two.

His general condition of health was excellent. No signs of vitamin deficiency were found. The thyroid gland was not palpable. The external genitals were normal

for his age; the same applied to skin and hair. — His mental development was normal.

His height was 86,5 cms. The tips of his fingers reached only to the iliac crest. The legs were very short and clumsy. His trunk seemed to be quite normal. The head was somewhat enlarged. The nose ridge was prominent. There was an abnormal mobility of his wrists. The hands were held in a valgus position. His thumbs were very short (*cf.* Fig. 2). The whole hand was of brachydactylous type. There was a moderate extension defect of the elbow joints and a slightly increased lumbar lordosis.

Summing up: A three years and eight months old boy with prominent disturbances of skeletal growth principally resulting in shortening of the limbs. There remains no doubt that this deviation is due to grave disturbances in the development of the chondrous skeleton.

No. 14. Mother of the propositus, A. A. E., born $^{30}/_{1}$, 1911. She had always been healthy. Had gone through three pregnancies with uneventful deliveries; had no abortions or stillborn. She was an intelligent woman and facilitated my communications with the rest of the large family in every respect.

At the examination in June, 1947, her height was 150 cms. Trunk and head appeared normal but a definite shortness of arms and legs was noticed. This deviation was by no means comparable to the gross aberration of her son but was nevertheless obvious. Especially the humeri and femora seemed to be affected. Her body type was short and plump with heavy musculo-skeletal elements. She informed me that she had grown very slowly as a child and had been definitely behind some of her sibs in this respect (*e. g.* especially in comparison with Nos. 7 and 8).

Her right hand was held in a slight varus position. Probably the radius was shorter than the ulna (deformity of Madelung?). Her hands were of a slight brachydactylous type with rather short fingers, especially the thumbs (*cf.* Fig. 2). No pathological signs from the internal organs were found.

Summing up: A 36 year old woman with slight but conclusive evidence of disturbed skeletal growth. Whether this deviation should be called pathological or not is a matter of taste. It is considered here as a mild manifestation of chondrohypoplasia.

Diagnosis: Chondrohypoplasia.

No. 32. J. H. E., born $^{13}/_{10}$, 1917, father of the propositus and first cousin of No. 14. He had always been healthy. As a child he had grown very slowly and his limbs had been observed to be a little shorter than normal.

At the examination in June, 1947, his height was 168 cms., which is slightly below the average in this family. He had an athletic body type with heavy musculoskeletal elements, very similar to that of his wife. The limbs are a little shorter than normal, most obvious was the shortness of the forearms and femora. His hands were short and broad of a slightly brachydactylous type. His general condition of health was excellent and nothing pathological was found from internal organs.

Summing up: In this case we find evidence of a slightly disturbed skeletal growth of the same type as in case No. 14.

Diagnosis: Chondrohypoplasia.

No. 81. R. U. E., born $^{23}/_{8}$, 1944, sister of the propositus. Normal delivery. She was breast-fed for 6 months and received cod liver oil. She was of the slow-grow-

ing type and definitely behind other children. At the examination in June, 1947, her height was 85 cms., which is considerably below the average (*i. e.* 92,2 cms.). There was a slight shortness of her limbs of the same type as in her parents.

Diagnosis: Chondrohypoplasia.

No. 82. M. R. K., born $^{19}/_{2}$, 1946, sister of the propositus. Normal delivery. She was very small at birth. Her growth had been of the same slow type as noticed in her sister. Her height was 70 cms. (June, 1947) against a normal average of 86,4 cms. In neither of these girls were signs of hypovitaminosis or other diseases found. Their development in other respects had been quite normal. In this last case any shortness of the limbs was hard to evaluate, but a disturbed skeletal growth seemed conclusive.

Diagnosis: Chondrohypoplasia.

FURTHER FAMILIES OF THE PEDIGREE.

The investigation was facilitated by the fact that the deviation here called chondrohypoplasia was well known to the members of this pedigree. They had long ago realized that some of them were short and clumsy and entirely different in body type from others who were thinner and taller. No. 80, however, was a type that was not known before.

Family No. 1.

No. 1. J. E. J. ♂, born $^{17}/_{7}$, 1824, died $^{16}/_{3}$, 1893 of pulmonary tuberculosis. He was only about 150 cms. high and had short limbs. His wife was tall with normal limb proportions.

Tentative diagnosis: Chondrohypoplasia.

No. 2. J. G. E. ♂, born 1862, died 1922 of pneumonia. About 160 cms. high with short limbs.

Tentative diagnosis: Chondrohypoplasia.

No. 4. A. V. E. ♂, born $^{19}/_{4}$, 1872, died $^{7}/_{7}$, 1929. According to his son, No. 32, his father was of the short type and very like himself.

Tentative diagnosis: Chondrohypoplasia.

Nos. 3 and 5, both women, deceased 1940, were described as tall and proportionately built.

Family No. 2.

No. 6. J. R. E. ♂, born $^{28}/_{1}$, 1890. He was born with relatively short limbs and had been of the slow-growing type. At the examination in 1947 his height was 160 cms. His body type was very similar to that of No. 32, with relatively short humeri and femora. His hands and fingers were short, broad and plump. He had been suffering from rheumatoid arthritis for several years.

Diagnosis: Chondrohypoplasia.

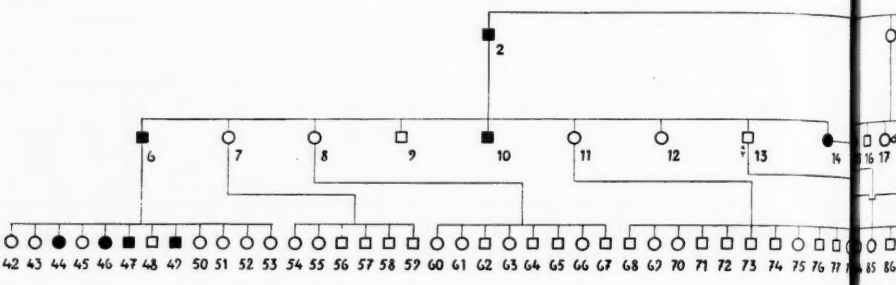
No. 7. J. E. R. ♀, born $^{30}/_{7}$, 1891. Not examined. See below.

No. 8. A. E. H. ♀, born $^{6}/_{3}$, 1895. Not examined. Se below.

No. 9. ♂, born $^{17}/_{10}$, 1897, died $^{3}/_{12}$, 1897, the cause of death unknown.

No. 10. A. E. ♂, born $^{28}/_{1}$, 1899. He had grown very slowly during infancy and childhood. Examined 1948: height 159 cms., the same body type as No. 32 but the shortness of his limbs was more prominent. He had short and plump hands.

Diagnosis: Chondrohypoplasia.



Scheme 1. Pedigree of the T-järvi family. Individuals with disturbed skeletal
positus, showing gr

No. 11. H. E. P. ♀, born $^{25}/_{5}$, 1901. Examined 1948. Her height was 156 cms. and her body type in some respects similar to that of No. 14. She was however proportionate and nothing deviating was known about her growth.

No. 12. F. I. E. ♀, born $^{7}/_{6}$, 1904, died $^{16}/_{3}$, 1905, cause of death unknown.

No. 13. H. E. ♂, born $^{24}/_{11}$, 1907. Examined in 1947. His height was 166 cms. with some very slight indications of the chondrohypoplastic body type. The diagnosis must be left open.

No. 14. ♀. See above, propositus' family.

Family No. 3.

No. 3. M. J. E. ♀, born $^{25}/_{10}$, 1864, died 1940 of heart disease. Described as tall, thin and proportionate. She had twelve children, of which the first one was illegitimate and is left out of consideration here. At her fifth delivery she had stillborn triplets. No information was available about these. Only three children of her marriage are still alive, Nos. 15 (J. H. F. ♂, born $^{22}/_{6}$, 1892), 22 (U. E. E. ♂, born $^{1}/_{2}$, 1902) and 23 (H. M. F. ♀, born $^{4}/_{8}$, 1904). At the examination in June, 1947 and Dec., 1948, none of them showed any signs of impaired skeletal growth. No. 15 was 175 cms. and No. 22, 172 cms. high and they were 55 and 45 years of age respectively. The rest of the sibs were all said to have been of the normal type.

Family No. 4.

No. 4. See above.

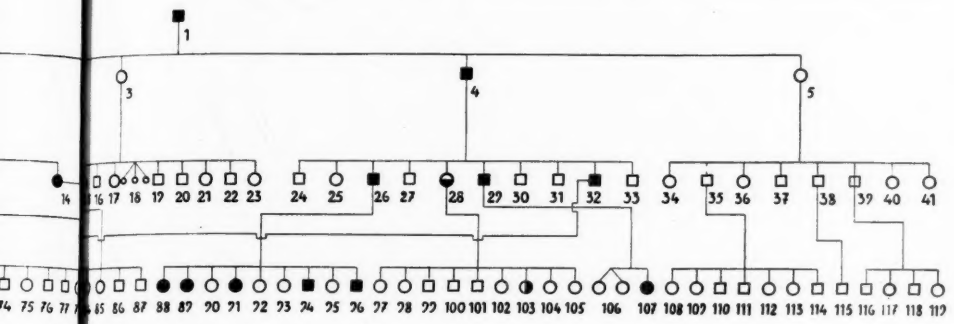
No. 24. A. H. E. ♂, born $^{2}/_{1}$, 1898, died $^{30}/_{10}$, 1899, cause of death unknown.

No. 25. H. S. E. ♀, born $^{6}/_{2}$, 1899, died $^{11}/_{3}$, 1907, cause of death unknown.

No. 26. A. H. E. ♂, born $^{22}/_{9}$, 1901, was very small at birth and later appeared to be of the slow-growing type. At the examination in 1947 his height was 160 cms. The limbs were relatively short, especially the legs, and he showed the same rather characteristic body type as No. 32. His hands were of the same short and broad type, with very short thumbs.

Diagnosis: Chondrohypoplasia.

No. 27. E. V. E. ♂, born $^{20}/_{2}$, 1904, died $^{15}/_{2}$, 1922 of meningitis. He was said to have been of the tall and thin type.



ed skeletal rohypoplasia) have been drawn black. □ = male. ○ = female. P = pro-
howing gr rophic disturbances.

No. 28. B. I N. ♀, born $^{31}/_{5}$, 1906. She had a gross congenital kyphoscoliosis. With this exception her growth during infancy and childhood was said to have been normal. At the examination in 1947 the limbs appeared to be of normal length. The question whether some chondrohypoplastic disturbances lay behind this deformity of her spine must be left open.

Diagnosis: Congenital kyphoscoliosis.

No. 29. S. V. E. ♂, born $^{31}/_{12}$, 1908, had been of the slow-growing type. At the examination in 1948 his height was 165 cms. His limbs were relatively short. The body type was similar to that of No. 32, only a little less square-built. His hands were of the same type and are shown in Fig. 3 in comparison with the normal hands of No. 72.

Diagnosis: Chondrohypoplasia.

No. 30. J. M. E. ♂, born $^{28}/_{11}$, 1911, died $^{1}/_{11}$, 1922, cause of death unknown. Probably of normal body type.

No. 31. O. H. E. ♂, born $^{20}/_{7}$, 1914, died $^{6}/_{5}$, 1922, cause of death unknown. Probably of normal type.

No. 32. See above, propositus' family.

No. 33. T. B. E. ♂, born $^{7}/_{8}$, 1921. Was said to be tall and thin, about 180 cms. high.

Family No. 5.

In this family all members were said to have been of the tall and thin type. This was confirmed for those still living, Nos. 35 (O. H. N. ♂, born $^{30}/_{4}$, 1898), 38 (J. V. N. ♂, born $^{6}/_{1}$, 1910) and 39 (J. O. L. ♂, born $^{5}/_{3}$, 1912). Their heights were 170, 173 and 178 cms. respectively.

Family No. 6.

No. 6. See above.

No. 44. B. E. ♀, born $^{13}/_{7}$, 1923. According to her mother, of the slow-growing and short-limbed type.

Tentative diagnosis: Chondrohypoplasia.

No. 46. H. E. ♀, born $^{27}/_{5}$, 1927, was examined in Dec., 1948. She showed the

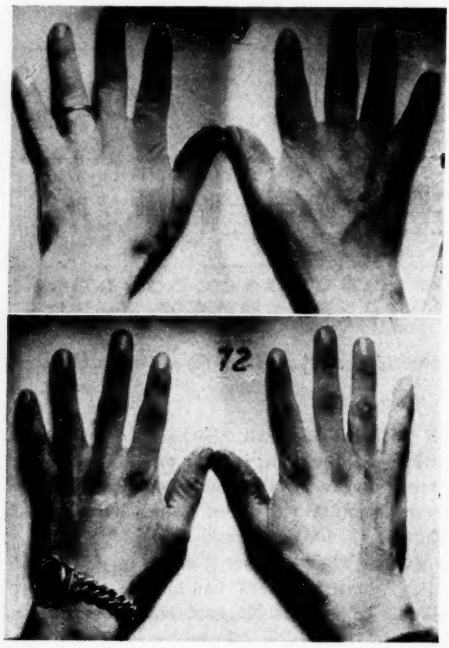


Fig. 3. Slight brachydactylous hand type of No. 29, ♂, who showed a chondrohypoplastic body constitution as compared with the normal hands of No. 72.

chondrohypoplastic traits rather pronounced. Her height was only 148 cms. and her limbs shortened, especially the tibiæ and fibulæ. Her body type was very similar to that of No. 14. Her hands were of a short and broad type, unlike that of her normal sisters (*cf.* Fig. 4).

Diagnosis: Chondrohypoplasia.

No. 47. E. E. ♂, born $^{16}/_{10}$, 1929. In June, 1947, his height was 156 cms. He had grown slowly and was now 1 cm. shorter than his two-year younger brother No. 48. His body type was of the musculo-skeletal square-built character, with somewhat shortened limbs. His hands were of the normal type, however.

Diagnosis: Chondrohypoplasia.

No. 49. A. E. ♂, born $^{6}/_{5}$, 1934. His appearance was very similar to that of No. 47. His height in 1947 was 132 cms., which is considerably below the average. He had grown very slowly, too. His hands were normal.

Diagnosis: Chondrohypoplasia.

Nos. 46, 47 and 49 (and by description No. 44) of this family constitute a deviating type as concerns body type and physical development. In this respect they are similar to their father. The sibs Nos. 43 (H. K. E. ♀, born $^{1}/_{12}$, 1921), 48 (K. E. ♂, born $^{14}/_{9}$, 1931), 50 (A. E. ♀, born $^{11}/_{3}$, 1936), 51 (E. E. ♀, born $^{11}/_{6}$, 1937), 52 (F. E.

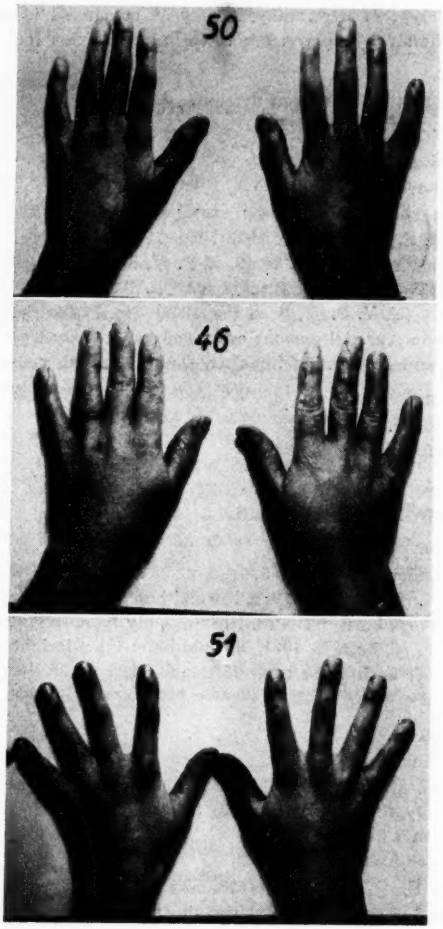


Fig. 4. Slight brachydactylous hand type of No. 46, ♀, as compared with two of her normal sisters.

♀, born $^{14}/_{11}$, 1939), 53 (M. E. ♀, born $^{20}/_{6}$, 1940), examined in 1947, too, were all of a body type within the normal range of variation. Nos. 42 and 45 by description have been considered as normal.

Family No. 7.

For geographical reasons it was not possible to examine this family, but some information has been obtained by correspondence and from the relatives in T-järvi. No. 7 wrote that she was tall and thin, her height 171 cms. All her children had grown normally and no obvious deviations had been observed. As she was well

aware of the short type of her family, it may be concluded with some confidence that no cases of chondrohypoplasia are to be found in this family.

Family No. 8.

No direct information has been available, but all members of this family are said to be of the normal type.

Family No. 11.

No. 11. See above.

The following children have been examined: No. 68 (A. L. P. ♂, born $^{5}/_{9}$, 1921), No. 69 (B. M. P. ♀, born $^{14}/_{1}$, 1923), No. 71 (B. V. P. ♂, born $^{2}/_{9}$, 1926), No. 72 (J. A. P. ♂, born $^{12}/_{9}$, 1927), No. 74 (S. E. P. ♂, born $^{12}/_{12}$, 1931), No. 75 (A. K. P. ♀, born $^{22}/_{4}$, 1933), No. 76 (K. M. P. ♂, born $^{5}/_{12}$, 1934), No. 77 (H. V. P. ♂, born $^{1}/_{6}$, 1941) and No. 78 (K. V. P. ♀, born $^{20}/_{6}$, 1944). No. 70 had left home and No. 73 died at the age of one year. All persons examined had a body type within the normal range of variation and no signs of impaired skeletal growth could be found.

Family No. 13.

No. 13. See above.

No. 79. No information.

Family No. 14.

Propositus' family, see above.

Family No. 15.

No. 15. See under family No. 3.

No. 83. J. H. F. ♂, born $^{6}/_{12}$, 1919. Normal body type. Height 177 cms. in 1947).

No. 86. K. J. F. ♂, born $^{12}/_{3}$, 1928. Normal body type. Height 168 cms. (in 1947).

No. 87. S. Ö. F. ♂, born $^{2}/_{4}$ 1931. Normal body type. Height 167 cms. (in 1947).

Nos. 84 and 85, both girls, born 1922 and 1925 respectively were said to be of the normal type.

Family No. 26.

No. 26. See above.

No. 88. L. R. E. ♀, born $^{3}/_{9}$, 1927, was of the slow-growing, rather characteristic short type. Especially the arms above the elbows were short. Height 151 cms. (in 1947). She had normal hands.

Diagnosis: Chondrohypoplasia.

No. 89. H. S. E. ♀, born $^{16}/_{10}$, 1928. She had the same body type as No. 88 and her height was 150 cms. (in 1947). The shortening of the arms above the elbows was more pronounced. Her hands and fingers were short, broad and plump.

Diagnosis: Chondrohypoplasia.

No. 90. S. I. E. ♀, born $^{15}/_{1}$, 1930. Normal body type. Height 165 cms. (in 1947).

No. 91. H. G. E. ♀, born $^{6}/_{3}$, 1937. She had grown slowly. In 1947 her height was 144 cms., and seen in 1948 she was shorter than the younger sister, No. 92. Her limbs were relatively short, her hands and fingers of the short and broad type.

Diagnosis: Chondrohypoplasia.

No. 92. M. E. E. ♀, born $^{6}/_{10}$, 1934. Normal body type. Height 141 cms. in 1947 and 148 cms. in 1948.

No. 93. G. G. E. ♀, born $^{24}/_{2}$, 1937. Normal body type. Height 127 cms. (in 1947).

No. 94. H. S. E. ♂, born $^{3}/_{9}$, 1939. Had the same body type as his father. He

had grown slowly and his limbs were relatively short, most marked in humeri and femora. His height was 110 cms. in 1947. His hands were short and broad with thick fingers.

Diagnosis: Chondrohypoplasia.

No. 95. G. I. E. ♀, born $^{25}/_{6}$, 1941. Normal body type. Height in 1947, 112 cms.

No. 96. S. B. E. ♂, born $^{19}/_{10}$, 1943. He was small at birth and had grown only a little. His height was only 91 cms. in 1947. The forearms and legs below the knees were shortened. His hand were normal.

Diagnosis: Chondrohypoplasia.

Family No. 28.

No. 28. See above.

No. 103. G. I. N. ♀, born $^{16}/_{10}$, 1940. She had a congenital luxation of the right hip joint. She had been thorougly examined at the Orthopædic Clinic in Härnösand in 1945, including X-ray (file No. 28 597).

Diagnosis: Luxatio coxæ sin. et subluxatio coxæ dext. congenit.

With the exception of No. 97, who was not at home, and No. 102, who died at the age of one year, the following children of this family have been examined: No. 98. (H. J. N. ♀, born $^{13}/_{8}$, 1930), No. 99 (S. B. N. ♂, born $^{15}/_{3}$, 1933), No. 100 (J. S. N. ♂, born $^{9}/_{9}$, 1934), No. 101 (P. L. N. ♂, born $^{11}/_{1}$, 1936), No. 103 (G. I. N. ♀, born $^{16}/_{10}$, 1940), No. 104 (E. G. N. ♀, born $^{7}/_{8}$, 1942) and No. 105 (D. S. N. ♀, born $^{6}/_{3}$, 1946). Their body types were all within the normal range of variation and nothing extraordinary had been noticed about their growth.

Family No. 29.

No. 29. See above.

No. 106. Stillborn twins, ♀♀, born $^{2}/_{8}$, 1943. No further information was available.

No. 107. M. B. M. E. ♀, born $^{10}/_{11}$, 1944. She was very small at birth and had grown only a little. In 1947 her height was 82 cms. and her limbs probably somewhat short.

Diagnosis: Chondrohypoplasia.

Family No. 35.

No. 35. See above. In this family Nos. 110 (S. O. N. ♂, born $^{6}/_{12}$, 1929), 111 (G. V. N. ♂, born $^{18}/_{2}$, 1932), 112 (G. G. N. ♀, born $^{8}/_{3}$, 1935), 113 (M. A. N. ♀, born $^{18}/_{5}$, 1937), and 114 (B. H. S. N. ♂, born $^{22}/_{2}$, 1940) have been examined. They had all grown normally and were of normal body type. The same information was obtained as for Nos. 108 and 109.

Family No. 38.

No. 38. See above.

No. 115. J. S. N. ♂, born $^{19}/_{3}$, 1936. Normal body type. Height in 1947, 150 cms.

Family No. 39.

No. 39. See above. All children have been examined and nothing exceptional was found: No. 116 (N. O. I. L. ♂, born $^{8}/_{10}$, 1939), No. 117 (E. L. O. L. ♀, born $^{7}/_{3}$, 1942), No. 118 (Å. J. L. ♂, born $^{14}/_{8}$, 1943) and No. 119 (B. E. L. ♀, born $^{6}/_{10}$ 1945). Furthermore, the wives or husbands of the following persons in the pedigree have been examined, *e. g.* Nos. 6, 11, 15, 26, 28, 29, 35, 38 and 39. They were all of a normal body type with no signs of chondrohypoplasia. No information has been obtained that other persons married into the family complex had any deviating body type.

CLINICAL DISCUSSION.

The lack of X-ray studies of the pedigree members will of course hamper an exact diagnosis. Nevertheless I think that the simple clinical observations made in this study may justify a genetical consideration.

The diagnosis of the propositus made by an expert radiologist was chondrodystrophy. The clinical signs and symptoms seem to fit in with this even if the picture is not quite classical. According to MØRCH (1941), however, all symptoms vary in degree from one individual to another. The following, in his opinion pathognomonic symptoms, have been found here: dwarfism, micromelia involving all four extremities, defective extension in the elbow joints, a slightly increased lumbar lordosis. This typical shape of the head, which occurs in 78 % of cases, was not present here. There was only a slight enlargement of the calvarium.

Whether the radiological picture should be considered as indicative of true chondrodystrophy may be a matter of discussion. As a matter of fact, with the discrepancy between radius and ulna, the diminutive fibulæ and the almost normal skull, it resembles closely two cases described by BRAILSFORD (1935: the H-family). In his family, too, the parents were both of small stature but showing no deformity of small limbs. They had eight children, four of which had short arms and legs and of these two were examined. BRAILSFORD named this type familial atypical achondroplasia and considered it different from true achondroplasia.

In the present case there is no doubt that the disturbances are of a chondrodystrophic nature. The definition seems to be more a question of academic interest, so I am leaving it open. However, the existence of different phenotypical types of chondrodystrophic disturbances seems established and it remains to show whether these also have a different genotypical background.

The characteristics of the members of the pedigree (drawn black in Scheme 1) who deviate from normality are as follows. Most of them have been observed to be very small at birth, although none of them was reported to have been premature. During infancy and childhood their growth is definitely retarded in comparison with their normal sibs. As adults they show a moderately reduced stature with a slight shortness of the extremities. The shortness of the limbs may affect humeri, forearms, femora or tibiæ—fibulæ more or less pronouncedly in different individuals. In most cases humeri and femora are shortened.

The trunk always appears normal (No. 28 may be an exception). The shape of the head shows no deviation from the normal range of variation. All affected individuals, however, are brachycephalic.

The body type of these individuals is rather characteristic and in most cases they are easy to separate from their normal relatives. They are square-built with heavy musculo-skeletal elements, whereas the other members of the pedigree are more gracile, thin and tall. The body type is reflected in some individuals in the type of their hands

TABLE 1. *Survey of height and body type of examined women in the pedigree.*

No. in the pedigree	Age in years	Height in cms.	Lower limit of height in Swedish population [1]	Body type
105	1	91	70,5	normal
82	1	*70*		chondrohypoplastic
119	2	76	75,4	normal
107	3	*82*	85,0	chondrohypoplastic
81	3	85		chondrohypoplastic
78	3	91		normal
117	5	109	98,0	normal
104	5	105		normal
95	6	112	104,0	normal
103	7	116	109,0	normal
53	7	112		normal
52	9	119	117,3	normal
113	10	129	121,0	normal
93	10	127		normal
51	10	122		normal
50	11	131	125,3	normal
112	12	142	130,9	normal
92	13	141	137,8	normal
75	14	167	142,8	normal
91	15	*144*	146,8	chondrohypoplastic
98	17	177	151,5	normal
90	17	165		normal
89	19	*150*	152,5	chondrohypoplastic
88	20	*151*	153,3	chondrohypoplastic
46	21	*148*		chondrohypoplastic
69	24	158		normal
43	26	164		normal
14	36	*150*		chondrohypoplastic
11	46	156		chondrohypoplastic?

[1] In the normal population only 0,5 per cent is below this figure (*cf.* BROMAN, DAHLBERG and LICHTENSTEIN, 1940).

TABLE 2. *Survey of height and body type of examined men in the pedigree.*

	No. in the pedigree	Age in years	Height in cms.	Lower limit of height in Swedish population [1]	Body type
	118	4	103	93,3	normal
	96	4	*91*		chondrohypoplastic
P	80	4	*86*		chondrodystrophic
	77	6	114	104,8	normal
	114	7	114	110,0	normal
	116	8	122	114,8	normal
	94	8	*110*		chondrohypoplastic
	115	11	150	127,3	normal
	101	11	136		normal
	76	13	146	133,9	normal
	49	13	*132*		chondrohypoplastic
	100	13	158		normal
	99	14	170	139,3	normal
	111	15	148	145,6	normal
	87	16	167	152,8	normal
	74	16	163		normal
	48	16	157		normal
	47	18	156	161,3	chondrohypoplastic
	110	18	164		normal
	86	19	168	162,5	normal
	72	20	182	163,2	normal
	71	21	174		normal
	68	26	174		normal
	83	28	177		normal
	32	30	168		chondrohypoplastic
	39	35	178		normal
	38	37	173		normal
	29	39	165		chondrohypoplastic
	13	40	166		chondrohypoplastic?
	22	45	172		normal
	26	46	*160*		chondrohypoplastic
	10	48	*159*		chondrohypoplastic
	35	49	170		normal
	15	55	175		normal
	6	57	*160*		chondrohypoplastic

[1] See note below Table 1.

and fingers. A short, broad and plump hand type was observed in 10 out of 17 examined persons with the signs just mentioned. This type is an intermediate one between the isodactylic and the normal hand.

It seems evident that all these individuals show some slight signs and symptoms of disturbed skeletal growth, namely of the chondrous

skeleton. In spite of the fact that it has not been possible to verify this by X-rays, I think it justified to consider this deviation tentatively to be of a chondrohypoplastic nature. With this reservation I have diagnosed the trait as *chondrohypoplasia* (RAVENNA, 1913, *op. cit.* BAUER, 1924).

As will be seen from Tables 1 and 2, the majority of the chondrohypoplastic individuals have a stature considerably below the average.

For those not aquainted with Swedish anthropology it should be noted that the present family comes from the Kalix district, where people are of a tall type comparable with the mean of the Swedish population, and a comparison with this mean seemed justified (*cf.* LUNDMAN, 1947).

Within the pedigree it was possible to compare the body height of 5 adults with chondrohypoplastic traits with 7 normal adults. The difference is significant (*cf.* Table 3).

TABLE 3. *Comparison of body height in men over 25 years of age with chondrohypoplastic and normal body type in the pedigree.*

Chondrohypoplastic		Normal	
No.	Height in cms.	No.	Height in cms.
10	159	35	170
6	160	22	172
26	160	38	173
29	165	68	174
32	168	15	175
		83	177
		39	178

Variance ratio 37,22 $P < 0{,}001$

A small stature combined with chondrodystrophic disturbances has been described previously by several writers, *e. g.* FARABEE (1905) in brachydactyly, DRINKWATER (1913) in minor brachydactyly, LIEBENAM (1939) in brachydactyly, and others.

GENETICS.

The pedigree is presented in Scheme 1. All individuals in whom signs and symptoms of disturbed skeletal growth were found have been drawn completely black. The facts presented would seem to favour the following interpretation.

The trait chondrohypoplasia is the effect of a single gene which

has its main action on the development of the chondrous skeleton. This gene probably has a high penetrance. The deviation from normality caused by it is admittedly slight, but it should be borne in mind that the conception of penetrance is always a function of our diagnostic ability.

The expressivity of this gene is evidently not topically limited to some special part of the chondric skeleton in the same way as has been observed in, for instance, the special types of branchyphalangy. The effect is moreover one of slight general impairment of the growth of precartilaginous bones, chiefly those of the extremities. Different bones are more or less involved in different individuals. The gene, however, always seems to affect the same biological process.

The mode of inheritance as estimated in Table 4 is one of simple dominance. There are no facts in favour of preponderance of either sex.

TABLE 4. *Statistical estimation of hereditary transmission and sex ratio. Included in this table are persons who have been examined as well as those diagnosed by the aid of anamnestic data.*

Families of which one parent is chondro-hypoplastic	Chondrohypoplastic children		Σ sibs
	male	female	
No. 1	2	0	4
No. 2	2 (+ 1?)	1 (+ 1?)	7
No. 4	3	0 (1?)	8
No. 6	2	2	12
No. 26	2	3	9
No. 29	0	1	1
	11 (+ 1?)	7 (+ 2?)	41

[1] Chondrohypoplastic children = 0,43 ± 0,08 (0,51 ± 0,08) %. Expected 0,50; $0{,}5 < P < 0{,}6$ $(0{,}9 < P)$. Proportion of males among the chondrohypoplastics = = 0,61 ± 0,11 (0,57 ± 0,11) %.

The question of the etiology of the propositus in this pedigree is of particular interest. His parents both have chondrohypoplastic traits and should reasonably be heterozygous for this »skeletal» gene. His two sisters show the same signs and symptoms of impaired skeletal growth. The most probable explanation is that the propositus is homozygous and that the effect of this condition is an increased effect on the development of the chondric skeleton. The result is a clear-cut pathological condition.

Principally this pedigree seems analogous to the wellknown family with brachyphalangy, of MOHR and WRIEDT (1919). In this family a

marriage between first cousins, both affected, occurred, *i. e.* both were heterozygous. One daughter of this marriage who died at the age of one year had her whole osseous system in disorder and was interpreted as a homozygote.

It is reasonable to assume that in the present pedigree we are dealing with a special genotypical entity within the group of chondrodystrophic disturbances. Furthermore it is important to realize that a »skeletal» gene which in heterozygous condition is perfectly harmless may, when occurring homozygously, have serious consequences.

It seems quite possible that the genes correlated to the chondrodystrophic disturbances may constitute a series of multiple allelomorphs which in their action differ quantitatively and qualitatively. From a eugenical point of view it seems worth considering that marriages between persons affected with even slight signs and symptoms of chondrodystrophic disturbances may involve a particular hazard, as for the prognosis of the children.

Case No. 28 with a congenital kyphoscoliosis and case No. 103 with a congenital dislocation of the hip joint remain to be considered. The occurrence of these individuals in the pedigree may be a mere coincidence or may have some connection with the chondrohypoplasia. This question must be left open. As neither of these persons shows any of the characteristic chondrohypoplastic signs otherwise so constantly found, and as all the sibs of No. 103 are quite normal, I am inclined not to believe in such a connection. It should be mentioned, however, that a premature ossification of the Y-cartilage may cause a dislocation of the hip (KAUFMANN, 1892), thus pathogenetically there may be a connexion between the two conditions.

SUMMARY.

(1) A pedigree has been studied of 123 persons in whom chondrohypoplasia is inherited as a simple dominant.

(2) Chondrohypoplasia is a slight impairment of skeletal growth with the following characteristics: retarded growth, a moderately reduced stature, a slight shortness of the extremities, a square-built body type with heavy musculo-skeletal elements, in most cases a hand type intermediate between normal and isodactylic. This condition, though being a clear-cut deviation from normality, is perfectly harmless.

(3) In a marriage between two affected individuals who were first cousins a boy was born who presented a serious pathological condition.

Most signs and symptoms point to a diagnosis of chondrodystrophy, probably corresponding to a special genotypical entity. He has been interpreted as homozygous for the »chondrohypoplastic» gene.

(4) The relation between different types of chondrodystrophic disturbances is discussed briefly.

(5) From a eugenical point of view it seems desirable to consider that marriages between persons affected with even slight signs and symptoms of chondrodystrophic disturbances may involve a particular hazard as regards the prognosis of the children.

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SOME EXPERIMENTS WITH TETRAPLOID TOMATOES

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INTRODUCTION.

FOR a number of years I have been engaged in the breeding of Swedish tomatoes, and when working with polyploid types I have made some observations of general interest which will be described here below. The present paper deals with investigations of different quantitative properties of tetraploid types as compared with the corresponding diploids and with a remarkable reciprocal difference observed in crosses between $4x$ and $2x$ materials.

Abundant literature is available on the effect of polyploidy on the morphological and physiological properties in different species. Principally I shall refer only to those parts of this extensive literature that concern the same problem or the same species as treated here.

MATERIAL.

The tetraploid tomatoes used in the experiments mentioned below were:

Tetraploid I: arisen spontaneously in a culture of Potentate;
» III: » » » » » » Carrick;
» IV and IX: produced by the decapitation-callus method (WINKLER, 1916) combined with colchicin treatment of plants of Danish Export;
» V: produced in the same manner in Kondine Red;
» VII: » » » » » » E. S. I (very probably this variety, although it is not absolutely certain).

In these experiments, of course, the corresponding diploids were cultivated parallelly. Occasionally also other material to be mentioned later in this paper was included.

The tetraploid types used here had been cultivated for 7—8 years, during which time they remained constant except for a few single aberrants which were not subjected to close examination. The fertility of the two spontaneous tetraploids (I and III) was poorer throughout than the others; some years it was even difficult to keep them alive.

COMPARISON BETWEEN TETRAPLOID AND DIPLOID POTENTATE.

A small-scale trial with the diploid variety Potentate and its tetraploid, each with 3 plots of 5 plants, was carried out in 1943 at Hammenhög. Since I left my position at the above-mentioned place a preliminary report of these experiments has been published by CARLSSON (1945) but they will be described here more fully. The ascorbic-acid content was determined by Dr. EMIL JOHANSSON, of the Horticultural Research Station at Alnarp, who also helped me to determine the number of chromosomes.

TABLE 1. *Comparisons between Potentate and its tetraploid.*

	Average 2x	Average 4x	Significance of differences
Yield per plant June—Sept., kg	5,47	4,40	**
» » » during June, kg	1,38	0,45	**
Nos. of fruits per plant	64	77	*
Gram per fruit	86	58	***
Gram seeds per kg fruit 3rd July	6,19	1,66	***
» » » » » 27th July	4,24	1,16	
(1.000 seed-weight) 3rd July, g	3,67	5,30	***
» » » 27th July, g	3,69	4,72	
Germinability, per cent	98,7	60,0	***
Dry-matter content 12th July, per cent	5,97	4,83	***
» » » 27th July, » »	5,43	4,85	
Ascorbic-acid content 20th July, mg per 100 g	19,1	20,0	None
» » » 29th July, » » » »	17,9	18,0	»
» » » 8th Sept., » » » »	18,7	19,6	»

Table 1 shows the main results of these experiments which were estimated by variance analysis. The differences in ascorbic-acid content are not significant, but otherwise there are great differences between the diploid and tetraploid types. These differences are of the same kind as those often found between diploids and tetraploids of other species.

Doubling of the chromosomes reduced the size but not the number of the tomatoe-fruits, so that the yield was smaller. The earlier crop was reduced most: the June crop of tetraploids was only 33 per cent of that of the diploids, but their total yield amounted to 80 per cent.

The fertility (g seed per kg fruit) was reduced considerably and this is probably the reason why the tomatoes were so small and the crops consequently meagre. Experience has shown that a more or less defective development of the tomato seeds will have a corresponding detrimental

effect on the growth of the fruit. In triploid plants, which regularly although rarely occur in tomato plots, I never found developed seed, and the tomatoes were very small (the average for triploid Lucullus, a variety normally producing rather large tomatoes, was for example 6,7 g). Reference is made to RICK (1945) for figures concerning the frequency of spontaneous triploids. The correlation between the number of developed seeds per tomato and the size of the tomato seems, however, only to hold good if the material includes the types showing genic or chromosomal unbalance — small number of seeds — small tomatoes. In the diploid *Lycopersicum esculentum* no such connection can be found. The varieties poor in seeds, especially the »fleshy» ones, often belong to those with the largest tomatoes.

If the number of seeds per fruit was low, the weight of the seeds was higher in tetraploids than in diploids, but the germinability was considerably lower.

The dry-matter content is significantly lower in tetraploids, as is most usual in autotetraploid forms. The test for determining the dry-matter content, which was made for every plot, consisted of a thin slice across the middle of the tomato. These determinations were made on 5 tomatoes from every plot (Fig. 1). KOSTOFF and AXAMITNAJA (1935) also found tetraploid tomatoes to have a lower percentage of dry matter than diploid ones (cit. from MÜNTZING, 1936).

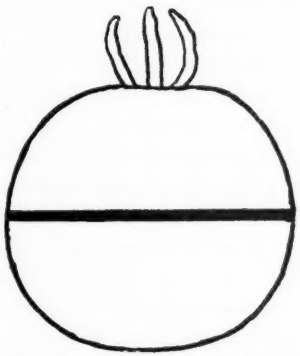

Fig. 1. The black line designates the slice used for determining the amount of the dry-matter content.

Most of the results which were obtained from this experiment, agree well with other results published on polyploid tomatoes. JØRGENSEN mentions that 40 cuttings of a certain triploid were completely sterile and says that »the tetraploids set regularly, but not so abundantly as the normal plants, and the fruits do not usually exceed normal size, being often smaller. This is connected with the lesser number of seeds contained in them» (JØRGENSEN, 1928). LESLEY (1928) also points out that he did not get seed from selfed triploids but »very sparingly» from $2x \times 3x$ and that they are »readily obtained» from $3x \times 2x$. According to LINDSTROM. observations of the usual *L. esculentum* show that »seed development cannot be classed as more (and often less) than fifty per cent normal. The tetraploid fruits, although numerous, are somewhat smaller than

those of the diploid from which these tetraploids were derived, asexually by the decapitation-callus method.» (*id.*, 1932.) On the other hand, *L. pimpinellifolium* MILL. (syn. *L. racemigerum* LANGE) which is quite compatible with *L. esculentum* was in its tetraploid form found to be very fertile (*l. c.*). SANSOME (1933) obtained on an average 21 seeds per fruit in tetraploids as compared with 115 seeds per fruit in the diploid type of *L. esculentum*. Also the reduced germinability of tetraploids has been mentioned here and there in the literature (JØRGENSEN, 1928; LINDSTROM, 1941; SANSOME, 1933).

In Swedish experiments also the poor fruit development of tetraploid tomatoes and its connection with the lowered fertility has been observed and pointed out by F. NILSSON (1948).

COOPER and BRINK (1945) express the following opinion as being the cause of the deficient fertility: »The tetraploid tomato produces considerably fewer seeds than the diploid, but this is due mainly to failure of many ovules to become fertile because of restricted pollen tube growth rather than to breakdown of seeds during development», an opinion which I cannot fully share, although for the present I have no evidence to disprove it.

During 10 years' observations LINDSTROM (1941) found an especially low fertility — with nearly half of the plants completely sterile — in a tetraploid obtained by decapitation from an originally haploid biotype ($x \rightarrow 2x \rightarrow 4x$). It is very interesting to note that a quadruplicated haploid is considerably less fertile than a duplicated diploid, just as in my experiments during the course of the years tetraploids I and III have been much more difficult to »keep alive» than the others, which are the results of callus- and colchicin-doubling (probably c-mitosis; LEVAN, 1938).

The ascorbic-acid content in the material examined by JOHANSSON (1939) was higher in tetraploid tomatoes than in diploid ones (46 as against 29 mg per 100 g), a result which »is in good agreement with those which were obtained by SANSOME and ZILVA 1933» (here translated from Swedish), a paper which I have not had an opportunity of studying. On the other hand, in my experiment (ascorbic-acid tests carried out by Dr. EMIL JOHANSSON) no significant differences could be observed, in spite of good agreement between the different determinations. These results do not suggest any definite regular connection between $2x$ and $4x$. A more recent experiment by CRANE and ZILVA (1949) showed, however, that environmental factors could affect the relation $2x$—$4x$ in the question of vitamin C considerably. They write: »Whilst there was no significant difference in the *L*-ascorbic acid content be-

TABLE 2. *Variation in some properties at different times.*

		G seed per kg		1,000 seed-weight		Per cent dry-matter content	
2*x*	3rd July	6,19	5,21	3,67	3,68	5,97	5,70
	27th July	4,24		3,69		5,43	
4*x*	3rd July	1,66	1,41	5,30	5,01	4,83	4,84
	27th July	1,16		4,72		4,85	
Total	3rd July	3.92		4,48		5,40	
	27th July	2,70		4,20		5,14	
Significance between 2*x* and 4*x*		***		***		***	
» » data		***		**		**	
» for interaction		**		**		**	

tween the tetraploid and diploid tomatoes grown in the sun, whether they were picked in a ripe or green condition, there was, however, in the tetraploid fruits (both ripe and green), a vitamin content markedly higher than in the diploid tomatoes when both were grown in the shade.» It ought to be mentioned that my material was cultivated in a hothouse.

As will be apparent from this survey of the literature my above-mentioned experiment did not yield many new results. Its real *raison d'être* is that so much numerical and statistical data could be collected in a single trial with 2*x* and 4*x* of one and the same variety, one of the commonest greenhouse tomatoes grown in Scandinavia.

It will be seen in Table 1 that a number of qualities were determined at two different times at an interval of approximately 3 or 2 weeks. A singular fact is that those changes observed after the interval were not the same in 2*x* as in 4*x*. As to the dry-matter content and the seed content of the fruit, both were considerably reduced in the diploid but not in the tetraploid, whilst the weight of the seed was much greater in the diploid than in the tetraploid. Remarkably enough, the relationship between the type and testing day is also significant (see Table 2).

COMBINATION OF DIFFERENT TETRAPLOIDS.

In 1945 a number of crosses were made between different tetraploid tomatoes in order to produce, if possible, combinations with a richer yield than in primary tetraploids. When judging the F_1 in 1946 it was noticed that all those estimated as the most successful crosses had tetraploid VII as one of their parents. Selection in F_2 and F_3 (1947—1948) was made mainly from these crosses.

As it was of interest to find out as soon as possible if the yield from tetraploid tomatoes could be improved by means of crossing, a small preliminary experiment was commenced in 1949 with those crosses that were available and their parents and, as far as possible, with corresponding diploids.

As the hothouse which I had at my disposal was also used for selection material after crosses between different types of diploids and variety trials with such material, the experiments with tetraploids had to be limited to small trials. The material was divided into 4 different experiments with 5 plants of each line in each trial, distributed at random like the plots in an ordinary field experiment. However, occasionally a plant was damaged during the course of the experiment so that it became necessary to treat the whole material as a single trial, when the analysis of variance was carried out. This procedure can hardly be objected to, because the areas used were very small and lay close together, and therefore the soil may be regarded as uniform.

The designations of the primary tetraploids were mentioned earlier. The diploid varieties used for purposes of comparison will here below be designated as follows.

P2: Potentate, of which I is a tetraploid.
C2: Carrick, of which III is a tetraploid.
D2: Danish Export, of which IV and IX are tetraploids.
E2: E. S. I, corresponding to VII, missing.
K2: Kondine Red. The tetraploid of Kondine Red (V) was not included in the experiment on account of mis-sowing.

The tomatoes were collected during the whole of June, July and August. At the last picking even the unripe tomatoes which were left were collected (the plants had been topped when at the level of the roof).

Tables 3—5 give a review of the results of this experiment, which also permits a comparison of the various primary tetraploids with the respective $2x$ varieties. We find that the fruit-setting, judged by the number of tomatoes per plant, is somewhat less in $4x$ than in the corresponding $2x$ except for IV and IX in comparison with D2. This applies also to I and P2, which did not show any difference in fruit-setting in the above-mentioned trial. On an average, the size of the fruit is smaller in $4x$ than in $2x$. The yield is always considerably decreased by the chromosome doubling, especially during the earlier part of the harvest, all in full analogy with the above-mentioned comparisons between P2 and

TABLE 3. *Data concerning fruit-setting and size of fruits in the combination experiment. Means.*

	No. of fruits per plant	Gram per fruit	*n*		No. of fruits per plant	Gram per fruit	*n*
P2	47	69	5	I × IV: 1	53	42	5
I	33	37	4	2	53	40	4
C2	54	60	4	III × VII: 1	43	39	3
III	36	45	5	2	45	40	5
D2	50	40	5	3	44	43	5
IV	46	26	5	4	50	39	4
IX	56	24	5	5	54	40	5
K2	54	59	5	6	55	44	5
V	—	—	—	V × VII	56	32	5
E2	—	—	—	VII × IX: 1	59	31	5
VII	53	35	11	2	56	37	5
(*n* = number of plants)				IX × VII	57	39	4

I. With regard to the total crop, which was treated statistically, a comparison of $2x$ and $4x$ showed the following:

Between	DF = 1	Variance = 22,39	Ratio = 86,12***
Within	» = 47	» = 0,26	

The various tetraploids also differ significantly from each other:

Between	DF = 5	Variance = 0,474	Ratio = 6,32***
Within	» = 24	» = 0,075	

TABLE 4. *Yield per plant in the combination experiment. Means and percentage respectively.*

	Kg June-Aug.	Kg June	Per cent June		Kg June-Aug.	Kg June	Per cent June
P2	3,28	1,36	41	I × IV: 1	2,14	0,83	39
I	1,20	0,36	30	2	2,24	0,88	39
C2	3,28	1,39	40	III × VII: 1	1,64	0,57	35
III	1,62	0,49	30	2	1,81	0,84	47
D2	2,01	1,22	61	3	1,91	0,89	47
IV	1,18	0,55	47	4	1,95	0,89	46
IX	1,35	0,63	47	5	2,18	1,28	59
K2	3,19	0,97	31	6	2,39	0,99	41
V	—	—	—	V × VII	1,79	0,86	47
E2	—	—	—	VII × IX: 1	1,85	0,93	50
VII	1,85	0,68	35	2	2,06	1,02	49
				IX × VII	2,23	0,88	39

Most of the crosses have in the best cases as many fruits per plant as the best of the parent tetraploids; only the two lines from I × IV are in this respect considerably better than I and IV. This also applies to the size of the fruit.

By the crossing and the subsequent selection the June crop has become much better than that of the parent plants. The delay in harvest time resulting from the doubling of the chromosome numbers of the different varieties can therefore be counteracted and considerably reduced by combination work.

TABLE 5. *Comparison between combination lines, parent tetraploids and original diploids.*

Line	Yield, per cent of the parents		Yield, per cent of the original diploids	
	a	*b*	*a*	*b*
I × IV: 1.....	178	181	65	106
2.....	187	190	68	112
III × VII: 1.....	101	89	50	—
2.....	112	98	55	—
3.....	118	103	58	—
4.....	120	105	59	—
5.....	134	118	66	—
6.....	147	129	73	—
V × VII	—	97	57	—
VII × IX: 1.....	100	137	—	92
2.....	111	158	—	105
IX × VII	165	120	111	—

Table 5 shows that most of the pedigrees obtained by repeated selection to all appearance surpass their parent tetraploids considerably.

The analysis of variance gives the following results:

$4x$ — cross lines	DF = 1	Variance = 4,16	Ratio = $16{,}64^{***}$
Within	» = 83	» = 0,25	

The variation between the different combination lines is also statistically significant:

Between	DF = 11	Variance = 16,51	$50{,}03^{***}$
Within	» = 43	» = 0,33	

The line showing the largest yield is III × VII: 6. The most important of the differences which can be produced with this as one member shows the following significance:

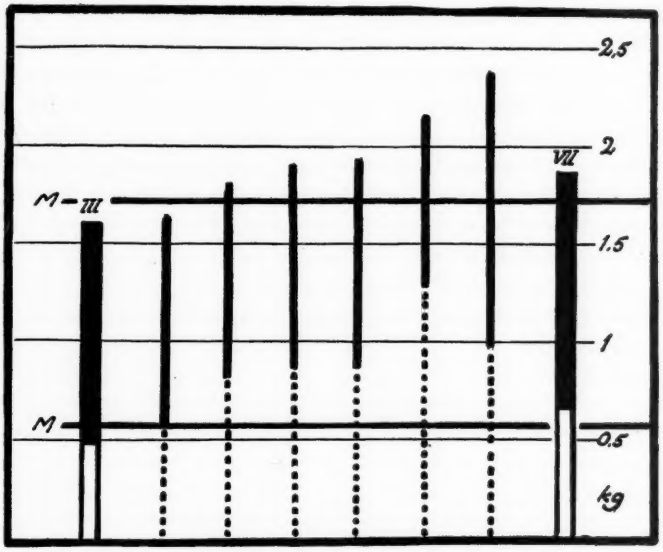

Fig. 2. Yield of III × VII lines and their parent tetraploids. The blank part of the columns respectively the dotted part of the line = yield during the first third of picking period.

III × VII: 6 — all other cross lines —
» — other III × VII lines *
» — III **
» — VII ***

The superiority of I × IV: 1—2 over I and IV is not statistically proved. But the difference between the best line of VII × IX (and reciprocally) on the one hand and IX on the other hand is significant (**), whilst the difference between the above-mentioned line and VII gives a P-value of approximately 0,05.

From these results we may conclude *that it was successful by means of selection in crosses between different »raw tetraploids» to obtain a yield which considerably and significantly surpassed that of the parents, and not least to diminish the delay in harvest following the chromosome doubling* (here the June crop).

If we then compare the cross-lines with their corresponding varieties of diploids from which they were obtained we find that the new lines give, as a rule, a result much inferior to that of the diploids. In the cross

I × IV, however, where a comparison can be made with the two original diploids the slight superiority of the cross-lines over the original diploid with the smallest yield was not significant. On the other hand, the original diploid with the best yield (P2) was but very slightly better than the cross-lines. The variance ratio here is 6,13; 4,75 is necessary for $P = 0{,}05$, and 9,33 is necessary for $P = 0{,}01$.

Already the fact that it was possible to obtain *some* combination line comparable to one of the diploid original varieties is rather satisfactory if it be borne in mind that selection was made only in two generations (F_4 lines were tried in this experiment).

Some observations but no numerical data on the increase in fertility in segregating material of tomatoes are to be found in the literature. SANSOME (1933) who experimented with »doubled» F_1 seedlings found that »later generations of the tetraploid tomato show more variation in fertility than the first (induced) tetraploid generation». LINDSTROM (1941) observed that several of what he calls allotetraploids (largely *L. pimpinellifolium* × *L. esculentum*, others not specified) were more fertile than those haploids which had been doubled twice, and with which he compared them (see above). Having pointed out the low fertility of tetraploid tomatoes as compared with diploids, LESLEY (1948) claims to have observed that »some varietal hybrids appear to be nearly as fruitful as the diploids», which, however, cannot be said of my experimental material. Finally, F. NILSSON writes (1948; here translated from Swedish): »Another way, which is also used for increasing fertility, consists of crossing different tetraploid types. By this method definite results have not yet appeared.»

TETRAPLOIDY AND BREEDING OF TOMATOES.

For judging the possibilities of obtaining useful results for tomato breeding by means of chromosome doubling a considerable experience from work with other plant species is a good asset. It would of course serve no purpose to discuss the voluminous literature on the subject, because it would involve too great a disproportion between the scientific apparatus and the scientific results. I will therefore deal mainly with some papers from the Cytogenetic Department at Svalöf. Some experiences of this institution, which since 1931 has been engaged with all Swedish agricultural plants, have recently been published in English (LEVAN, 1948; MÜNTZING, 1948). LEVAN's publication also contains a

complete list of references of the work published by the Department (103 Nos.).

General experience has shown that no newly developed polyploids — »raw polyploids» — are immediately useful in practice. They suffer from various defects and are, as a rule, more or less sterile. They also nearly always develop slower than the original types. It is also a general experience that polyploids cultivated from different varieties of the same species can differ considerably in many respects. This was also found to be the case in the author's experiments with tomatoes referred to earlier.

Some authors attribute the undeniably lower values of experimental polyploids solely to chromosomal irregularities. These irregularities are however hardly so general or so great that they can explain the general reduction in breeding value characterizing raw polyploids. MÜNTZING and LEVAN think the most probable reason is that the increase in the number of chromosomes causes a disturbance of the physiological balance necessary for the welfare of the plant. »A doubling of the chromosome number undoubtedly involves a serious disturbance of the genotypical balance, and it is therefore not at all surprising that our new polyploids suffer from certain defects» (LEVAN, 1948).

The bare fact that so many cultivated plants are polyploid argues in favour of the practical value of these types. Our forefathers naturally chose the most suitable cultivation material from that available in the form of wild flora or cultivated types. That the material that has gradually been »approved» consists mainly of polyploids suggests that polyploids have certain characteristics favourable for breeding purposes.

Both diploids and forms with different grades of polyploidy are seen in wild flora. Knowledge of the physiological and ecological relations between these two groups may be of great importance when judging the breeding value of polyploids. An excellent all-round, detailed, and clear critical survey of what is known of wild polyploids, of polyploids long since bred, and of experimental polyploids as well as a detailed discussion of this matter has been published by MÜNTZING (1936). Besides this, GUSTAFSSON (1948) grouped the wild flora from different points of view and with regard to the effect of polyploidy. MÜNTZING comes to the conclusion that intra-specific chromosome races and autotetraploids react in the same way in many respects. This means that one can really draw conclusions of practical value from the behaviour of the wild polyploid races. The autopolyploid races and even experimental autopolyploids have been found to react in an entirely different way to

environmental conditions (drought, cold, nourishment supply, *etc.*) than do the diploids.

As regards tomatoes, CRANE and ZILVA (*l. c.*) have shown »that when the plants are deprived of direct sunshine during growth, the *L*-ascorbic acid content of tetraploid tomatoes is not so much influenced as is that of diploid plants». In this way the changed reaction norm ought to cause the tetraploid races to seek different places to grow in than do the diploid plants, and it has also been noticed that »chromosome races are ecologically different» and further that »the races have definitely or probably different geographical distributions». (MÜNTZING, 1936.) Chromosome races are, as a rule, more resistant to unfavourable influences (*cf.* CRANE and ZILVA, *l. c.*, concerning the experimental $4x$ tomatoes) and more »plastic».

As far as tomatoes are concerned, one of the conclusions one ought to be able to draw of this is that if one succeeds in obtaining tetraploid plants more or less capable of being cultivated, it is not sure that they will show maximum yields when grown in a hothouse, which is at present the form most often used by commercial tomato production in Sweden. It is feasible that it will not be possible to produce an autotetraploid tomato suitable for this manner of cultivation but nevertheless one which is far superior in a cold greenhouse. Or it may be that these tetraploid varieties *in spe* react in a different manner to manuring with nitrogen, potash, phosphoric acid, *etc.*

The big drawback with autotetraploid tomatoes is, as pointed out above, their very poor fertility; in this respect they resemble other experimental »raw polyploids», whilst polyploid chromosome races are almost always completely fertile (MÜNTZING, 1936). As mentioned earlier, the poor fertility of the primary autopolyploids is believed by experienced researchers to be due to a multiplication of the chromatin mass with resulting disturbance of the genic balance and thereby of the physiologic balance of the plant. Chromosome races »are the successful survivors from a much larger material (MÜNTZING, 1936) and »every spontaneous species, wild or cultivated, diploid or polyploid, is the result of a long process of evolution» (LEVAN, 1948). Therefore these old polyploid races do not show the disturbed balance and the consequential sterility which characterize the experimental polyploids.

It has been proved that it is much easier experimentally to approach the perfect balance in chromosomal races of allogamous than of autogamous plants. This must be due to the fact that the material of an allogamous plant will rather soon be thoroughly intercrossed, and that it

is the most fertile among the great number of combinations formed that will be preponderant in the total seed crop.

As far as tetraploid tomatoes — as well as other autogamous plants — are concerned, attempts should be made artificially to copy what happens in nature in a population of tetraploid allogamous plants, if quick results are to be obtained. This may be done by crossing $4x$ varieties with each other and then gradually selecting the best — in the beginning especially the most fertile — lines. It is therefore not difficult to copy the methods of nature, but it is all the more difficult to obtain the quantities like those obtained in nature both as regards time and frequency. By »doubling» as many different tomato materials as possible and by making a maximum number of crosses in the $4x$ material thus obtained, some degree of success may be expected. MÜNTZING (1948) was able to increase the yield of tetraploid barley by selection in segregating populations, and barley may be compared directly with tomatoes both as regards its property of pronounced autogamy and because its breeding value is dependent to a great extent on its fertility.

The author's experimental experience described above shows that different primary materials of tetraploid tomatoes may differ significantly, so that there is reason to procure more of such material of different kinds, and, secondly, that by a selection from the results of crossings, the plants may already after two segregating generations be considerably better than the raw tetraploids both as regards yield and earliness, the two valuable properties which suffer most by doubling of the chromosome number. That it was not possible for me after selection from two generations to reach the level of the best diploid original varieties is not to be wondered at, because what nature has produced in the course of a very long period of time with an almost unlimited material can hardly be expected to be performed artificially by man in almost no time.

An important question is whether selection in a cross between two tetraploid tomato plants can reach a level equal to that of the best diploid original variety. Plant breeders are often of the optimistic opinion that as old cultured plants, which are now the best, are often polyploid, and as chromosome races in nature have as a rule proved best, theoretically the most promising course is given. But, in my opinion, one cannot *a priori* regard the objection as unjustified that nature in this way of evolution has perhaps already reached an optimum, or, in other words, that if it is possible to make progress by polyploid breeding, it would have already been done by nature.

GUSTAFSSON's (1948) paper on the relationship of polyploidy to

ecologic factors in nature is very satisfactory to read for a plant-breeder. GUSTAFSSON's results are best described in his own words: »Man's society apparently favours polyploidy. This becomes evident from the analysis of hemerophilous [1] and hemerophobous elements within one and the same flora region. Culture conditions contribute, I think, to the change of annual diploids into polyploid weeds. Presumably the diploid predecessors were not able to create successful (agro-)ecotypes, or these could not compete with the autoploid and amphiploid products» (*l. c.*, p. 21).

That is is the ecological factors connected with culture that are also those which favour polyploidy, can constitute a strong objection against the above-mentioned possibility that nature has already reached optimum or nearly reached it in the polyploidization process. Because, even if this be true as regards plant populations not influenced by culture, it is less probably true as regards the ecological environment influenced by human cultivation, which is relatively young. This ought to apply to agro-ecotypes approximately to the same extent as to pure cultivated plants. In addition hereto, the agronomic conditions we now offer cultured plants are quite different on account of our manuring methods and many other factors which did not exist during older (and really not so very old) cultural eras. One might therefore say that nature has only advanced a short distance along the course we are pursuing experimentally, because the selecting factors constituted by cultural conditions have only existed a relatively short time.

Having pointed out that the poor fertility among experimental autotetraploids »is probably the most important limiting factor in their practical utilization», RANDOLPH (1941) says: »But a previously stated superiority in other characteristics may outweigh the deleterious effect of reduced fertility». If fertility is the limiting factor in any cultivated plant, it is especially so in the tomato, whose fruit development and thereby yield is to a high degree dependent upon its fertility. Whether $4x$ tomatoes have any other advantageous characteristics making their breeding worth-while even though it should not be possible by means of increased fertility for them to obtain a yield greater than of the diploid types, is a question to which I cannot give a definite answer. It seems, however, as if they were still healthier when the diploids begin markedly to age and as if they have better table-qualities than the diploids.

[1] »Species which profit from culture with regard to their occurrence and spread» (*l. c.*).

The great plasticity characterizing polyploid races (MÜNTZING, 1936) may also be expected to characterize autotetraploids (as mentioned earlier) and might then be one of the valuable factors acting as substitute for fertility referred to by RANDOLPH. CRANE and ZILVA's previously mentioned experiments on the vitamin-C content of $2x$ and $4x$ tomatoes also point in this direction. In my experiments there is another similar example. The dry-matter content falls considerably from the 3rd to the 27th July in the $2x$ type but not in the $4x$. This also applies to seed production (the contrary being the case with the size of the seed).

The collection of tetraploid tomatoes varying as much as possible as regards desirable properties means a completely new variation range with regard to the polygenes (*sensu* MATHER) also influencing the economic cultivation value. If it is then possible to bring this complicated and sensitive system into genic and physiologic balance, there should be good prospects of obtaining the desired results. LESLEY (1948) in his review of breeding problems of tomatoes is also rather hopeful: »Tetraploid tomatoes have not so far proved to be of economic importance but the possibility remains that fruitful types may be developed which will have special, desirable properties.»

DEVELOPMENT OF THE FRUIT IN RECIPROCAL CROSSES BETWEEN 2x AND 4x.

In 1946 my son, SVEN-ERIK NILSSON, carried out some reciprocal crosses between $4x$ and $2x$ tomatoes. It was already known from the literature that crosses between tetraploid and diploid tomatoes are sterile (JØRGENSEN, 1928; LINDTSROM, 1922; LINDSTROM and HUMPHREY, 1933). WINKLER (1916), publishing his paper before the fruit was ripe, observed »rascher Fruchtansatz» in $4x \times 2x$ and therefore thought he might obtain the triploid form, but was naturally unsuccessful.

In the above-mentioned small experiment 16 fruits were obtained from $2x \times 4x$ and 12 fruits from $4x \times 2x$. Each tomato was weighed separately. A considerable difference was found in size between the fruits of the reciprocal crosses. The mean weight per tomato for $2x \times 4x$ was 23,13 and for $4x \times 2x$ 56,67 g. The χ^2-analysis which I made showed the difference to be significant.

As I found this reciprocal difference interesting, I decided to examine it on a larger material. The crosses I tried were

Potentate × its tetraploid and reciprocally
Carrick × » » » »
Lines from (Kondine Red × Carrick) × various 4x cross lines and reciprocally
Potentate × ditto
Carrick × ditto

The last combinations had to be resorted to because the other material did not produce flowers enough for so many crosses as I wanted to make. All the 2x lines used in this experiment were, however, characterized by an approximately uniform size of the fruits which *mutatis mutandis* also applies to the 4x lines (see above). It was also found when grouping the material, which was naturally weighed and recorded separately for every group, that the groups varied in a uniform manner for which reason they were united in the χ^2-analysis.

On closer study of the literature I noticed that this phenomenon had already partly been observed (KOSTOFF and KENDALL, 1934; COOPER and BRINK, 1945), but as I had a larger and numerically more detailed material than these researchers I continued the investigation.

None of the fruits examined had any fully developed seeds. RICK (1946) makes a distinction between two types of abortion in genetically unfruitful tomatoes, *the collapsed type*, »probably conditioned by adverse chromosomal unbalance of the gametophyte itself» and *the substitution type* with lacking differentiation of the megaspore mother-cell. The sterility attending polyploidy is a consequence of the first-mentioned type of abortion (see also COOPER and BRINK, 1945).

The number of pollinated flowers in this experiment was 329 and, as will be apparent from the following scheme, the fruit-setting of the two reciprocal crosses differed considerably.

	Fruit	No fruit	n	Percentage fruit-setting
2x × 4x	47	131	178	26,40
4x × 2x	74	77	151	49,01

$\chi^2 = 17{,}942^{***}$

Strangely enough, KOSTOFF and KENDALL (1934) and COOPER and BRINK (1945) who worked with *L. pimpinellifolium* obtained no ripe fruits after 2x × 4x. One might imagine that the fruit-setting I obtained in this combination was due to spontaneous 2x × 2x. However, emasculation and pollination was carried out very carefully by the author himself and — the definite proof that the fruits obtained did not result from 2x × 2x — all of the fruits were quite sterile. As to the method, it should be mentioned that pollination was carried out immediately after emascul-

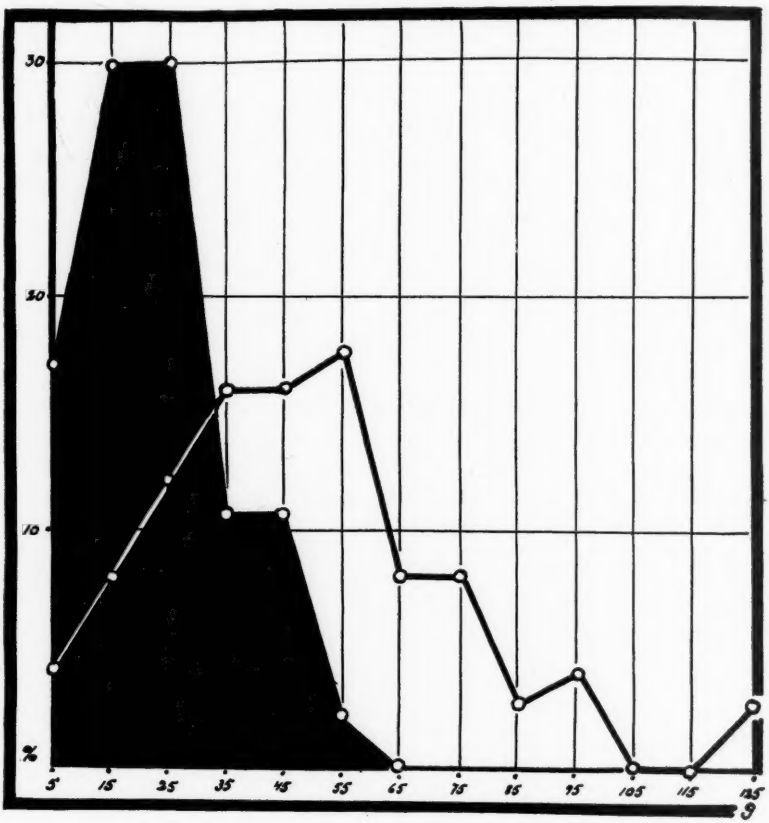

Fig. 3. Variation in the size of fruit of $2x \times 4x$ (left curve) and $4x \times 2x$ (right curve). Absciss: class centres.

ation and I used flowers in which the petals had just or hardly sprouted but not become yellowish, which, judging by my own experience, seems to give best fertility.

The development of the fruit after reciprocal crossing will be apparent from the following scheme and from Fig. 3.

	n	Mean g per fruit	
$2x \times 4x$	47	22,45	Difference***
$4x \times 2x$	74	47,97	

A remarkable result, however, which was observed only in a small material and will therefore not be discussed in detail here but which is worth new experimental investigation, is that $4x \times$ the very small fruited *L. pimpinellifolium* gave just as large if not larger fruits than $2x \times$ *L. pimpinellifolium*.

	n	Mean g per fruit	
$2x \times$ *L. pimp.*	10	40,00	Difference not significant
$4x \times$ » »	21	45,95	

This is not unlike COOPER and BRINK's results obtained when crossing $2x$ with $4x$ *L. pimpinellifolium* and $2x$ *L. peruvianum* (*l. c.*). They found as follows

$2x$ *pimp.* $\times$ $2x$ *peruv.*: seeds collapse,
$4x$ » $\times$ $2x$ » : many seeds grow to maturity

In COOPER and BRINK's experiments two different species were used, whereas *L. pimpinellifolium* and *L. esculentum* can hardly be regarded as being different species; they are at any rate fully fertile when crossed with each other. It should, however, be pointed out that, according to LINDSTROM (1932), $4x$ *pimpinellifolium* is very fertile and has larger fruits than its diploid and is therefore in both respects very different from $4x$ *esculentum*.

It is difficult to find an explanation which will in every respect satisfactorily explain the great differences between the crosses $2x \times 4x$ and $4x \times 2x$. The genotypical properties of the embryo cannot be held responsible here, because it is the same in both cases, namely $3x$.

One is tempted to assume that $4x$ has in itself a greater tendency towards parthenocarpy than $2x$. But then one might expect that the triploid fruits, which are always sterile, would be intermediate in this respect. But they are instead still smaller than either $2x \times 4x$ fruits and $4x \times 2x$ fruits (triploids of varieties of approximately the same fruit-size as those used here: 6—7 grams per fruit). As showed histologically by COOPER and BRINK (*l. c.*), the seeds of $4x \times 2x$ develop much further before they collapse than do $2x \times 4x$. With our knowledge of the fact that the development of the tomato fruits is dependent upon the development of the seeds (see above), it will be understood that also this factor must be at work even if $4x$ really has a greater tendency to parthenocarpy than $2x$. The degree of parthenocarpy of $2x$, $3x$, and $4x$ can be examined comparatively by artificial production, *e. g.* treatment

with β-naphthalene acetic acid or the like, which, however, as far as I know, has not yet been done.

By rights one might think it quite natural that a tetraploid tomato should produce larger fruits than a corresponding diploid, because it constitutes as a whole an »enlargement» of the diploid. If $4x$ were fully fertile, its fruits should therefore be larger than those of $2x$. However, $4x$ is to a very high degree sterile as compared with $2x$, for which reason one does not know anything about the mutual relationship between the two types when of the same degree of fertility. As already mentioned, the results which LINDSTROM (1932) obtained by doubling of *L. pimpinellifolium* argue in favour of the case being as described above. In this case $4x \times 2x$ even with just as early a seed collapse as $2x \times 4x$ ought naturally to have larger fruits than the latter, a difference which is, however, increased by the increased longevity of the embryos in $4x \times 2x$.

As to the small size of the fruits of triploids, it need not necessarily argue against a greater capacity of parthenocarpy in $4x$ than in $2x$, because it is not impossible that triploidy in itself causes an inferior development of the fruit, and, besides, it is feasible that the embryo development is interrupted much earlier in triploids than in these crosses.

Furthermore, LESLEY (1928) found the case in reciprocal crosses between triploid and diploid tomatoes to be similar to that in those referred to above as regards tetraploids $\times$ diploids. But he did not mention the size of the fruit, in addition to which triploid crossed with diploid, in contrast to tetraploid crossed with diploid, really does produce seeds:

$2x \times 3x$: seed was produced »very sparingly»,
$3x \times 2x$: » » »readily obtained».

A common and well-known fact when crossing tetraploids and their diploid origin is that the pollen tube growth is stronger in connections between $4x \times 2x$ than in $2x \times 4x$ (see, *e. g.*, MÜNTZING, 1933). The ratio between the amount of chromatin in the style and in the pollen tube in the reciprocal crosses is therefore very different.

$2x \times 4x.$ $\quad 24 : 24 = 1 : 1$
$4x \times 2x.$ $\quad 48 : 12 = 4 : 1$

As many of the seeds develop more or less before they die, *i. e.* are evidently fertilized, the varying growth capacity of the pollen tube in

the reciprocal crosses cannot explain altogether the difference observed in the size of the fruits.

Attention has long since been given to the fact that the sterility of crosses of this type may depend upon unsuitable relationship between the embryo and the tissues enclosing the latter. Whilst some researchers paid special attention to qualitative differences, MÜNTZING (1930 b) made it probable that the quantitative chromosome relations between endosperm, embryo, and surrounding maternal tissues play the greatest and commonest rôle, an opinion accepted and, as far as tomatoes are concerned, elaborated by KOSTOFF and KENDALL (1934) and COOPER and BRINK (1945).

MÜNTZING (1930 a) who created the distinct and unequivocal expressions »haplontic» and »diplontic» sterility, both expressions used far too seldom in manuals on the subject, believes that autotetraploid sterility is often largely diplontic, which is also pointed out by LEVAN (1945). In the material dealt with here the difference between the reciprocal crosses cannot depend on haplontic sterility because it is caused by different growth of identical embryos (namely $3x$). As to LESLEY's (1928) results which he obtained with $3x \times 2x$ one might expect also haplontic sterility, because the constitution of the embryo in the reciprocal crosses is

$$2x \times 3x = x + (x - 2x)$$
$$3x \times 2x = (x - 2x) + x$$

and there is every reason to believe that the gametic elimination is different in the two sexes.

The ratios between the quantities of gene substance in endosperm, embryo, and somatic tissues in the present material were as follows:

	End.	Emb.	Som.	End. : Emb.	End. : Som.	Emb. : Som.
$2x \times 4x$	4	3	2	1,33	2,00	1,50
$4x \times 2x$	5	3	4	1,67	1,25	0,75
$2x \times 2x$	3	2	2	1,50	1,50	1,00
$4x \times 4x$	6	4	4			

In support of his opinion that the quantitative differences as regards the number of chromosomes are the most important in cases like these, MÜNTZING (1933) points out the known fact that the amount of chromatin influences the size of the cell and therefore very probably also influences physiological conditions, which in turn naturally influence the development of the seed. As to the influence of the different ratios he thinks,

unlike researchers who believe the ratio embryo: endosperm to be the most essential, that »it seems much more natural to ascribe the difference to the chromosome number of the mother plants».

As to the reciprocal crosses of tomatoes, KOSTOFF and KENDALL (1934) are inclined to regard the ratio embryo: endosperm as the most important, whilst COOPER and BRINK (1945) share MÜNTZING's opinion. After detailed histological studies they came to the conclusion, however, that the embryo does not play any great rôle in this connection but that it is the relationship of the endosperm to the somatic tissue that is decisive. »The evidence is clear in the writer's judgement that the embryo as an agent contributing to the present type of seed failure can usually be ignored. At most its rôle can be only secondary to that of the larger, more active endosperm. It is the later tissue in its inter-relationships with the maternal portion of the young seed that determines primarily whether development succeeds or fails» (*l. c.*). — In the above review it is apparent that the relationship deviating least from the normal is endosperm: embryo, so that there is hardly reason to ascribe any essential influence to it, whilst the relationship between the endosperm and the embryo, on the one hand, and somatic tissues, on the other hand, deviates much more from normal. In the decision which of the inter-relations embryo : soma or endosperm : soma plays the greater rôle COOPER and BRINK's opinion, which is based on thorough investigations, should be taken into account. One cannot, however, *a priori* claim that what holds good for tomatoes will also hold good for all other plants or even for all other dicotyledones.

It is now generally believed that the pollen stimulates the growth of the fruit over and above that following upon its fertilization of the egg-cell and the increasing size of the fruit occurring parallel with the growth of the embryo. This stimulation is due to the fact that during germination and growth the pollen contains and produces growth hormones. Under these circumstances it is not unreasonable to think that pollen of $2x$ plants contains or produces more growth hormones than pollen of $4x$ plants and therefore exercises a stronger influence in this direction on the embryo and on the fruit than $2x$ pollen tubes from the tetraploid. Such a hypothesis is in good agreement with the fact that $3x \times 2x$ gives better fertility than $2x \times 3x$. If it can be assumed that the possibly higher auxin-content of $2x$ pollen also influences its own growth in the style, this might also explain the generally known fact that the pollen tube growth occurs more readily in the combination $4x \times 2x$ than in $2x \times 4x$. The hypothesis of KIHARA and NISHIYAMA discussed in detail

by MÜNTZING (1933) seems in certain respects to be similar to that above, but on closer examination it will be found to differ essentially, which will not, however, be discussed here. It is probably not impossible to examine experimentally whether $2x$ pollen in tomatoes does really give stronger hormonal growth stimulation than $4x$ pollen. The treatment of the emasculated flowers with extracts of the respective types of pollen ought by the induction of stronger or weaker parthenocarpy give an answer to the question.

Just because the inferior fertility associated with polyploidy largely is of diplontic type one ought not to deny that it may sometimes be haplontic. This has already been mentioned in the discussion of LESLEY's reciprocal crosses between triploid and diploid tomatoes and is also apparent from the briefly mentioned crosses between *L. pimpinellifolium* and *L. peruvianum* examined by COOPER and BRINK (*l. c.*). They obtained the following results:

$4x$ *pimp.*	× $2x$ *pimp.*:	no	developed seeds
$4x$ »	× $2x$ *peruv.*:	many	» »
$2x$ »	× $2x$ » :	no	» »

With $4x$ *pimpinellifolium* as mother a species-foreign *peruvianum*-genome — but not a *pimpinellifolium*-genome of identical type — can result in complete development of seeds contrary to what happens when $2x$ *pimpinellifolium* is the mother. Here there must be some interaction between genic and physiologic factors.

It should be mentioned perhaps that with our present knowledge of the fertility of tomatoes it may be assumed that the triploid plants occurring spontaneously in almost every big plot of tomatoes are fruits of a combination between an unreduced egg-cell and a reduced male gamete and not the contrary.

It is apparent from this review that it is difficult to find any genic or physiologic condition which can by itself explain the results of the reciprocal crossing results described above. As to the physiological side of the question, it might be presumed that changes in pollen tube growth, unbalance between the tissues of the ovule and possibly differences as regards parthenocarpy and hormone production interact to give the result which was to be explained.

SUMMARY.

In a trial with the tomatoes Potentate and its tetraploid, the tetraploid was found to have smaller fruits, a smaller yield, later ripening,

less seed production, lower dry-matter content, and inferior germinability than the original variety.

By crossing different tetraploid tomatoes it was possible already after selection in two generations to produce plants superior in yield and earliness to the parent tetraploids but it was not possible to surpass or even generally reach the level of the original diploids.

The possibility of obtaining valuable breeding results with polyploid material is discussed on the basis of our knowledge of the effects of polyploidy in other plant species.

The cross $4x \times 2x$ gave a considerably better fruit-setting and larger fruits than the cross $2x \times 4x$, a fact which is compared with those of a similar nature reported in the literature. The different possibilities of explaining this phenomenon are discussed.

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ON THE C_0 AND C_1 GENERATIONS IN ALNUS GLUTINOSA

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THE fact that colchicine treatment primarily results in mixoploidy has for long been known (see, *e. g.*, GYÖRFFY, 1940; JOHNSSON and EKLUNDH, 1940; SATINA and BLAKESLEE, 1941). In polyploiding experiments with herbaceous plants less attention is paid to the constitution of the C_0 generation. Treated plants or organs flower a few weeks or months after the time of treatment and polyploid C_1 progenies can be raised. On the other hand, in experiments with perennial ligneous plants, which do not produce flowers from treated tissue until after the lapse of several years, the behaviour of the C_0 generation comes into prominence. Almost all earlier reports on colchicine treatment of woody plants, *e. g.* MIROW and STOCKWELL (1939), JOHNSSON and EKLUNDH (1940), JENSEN and LEVAN (1941), have in fact concerned C_0 products. The view has been advanced (STRAUB, 1941) that the colchicine method is not applicable to such plants because the polyploidy induced by the treatment is thought to be lost during the long period, with its innumerable cell generations, that passes between treatment and flowering. However, there are means of ensuring the continued existence of the polyploid tissue. Unaffected shoots can be removed from the C_0 individual in order to favour the affected, and affected buds can be isolated from affected branches by inoculation (JOHNSSON and EKLUNDH, 1940; DERMEN and BAIN, 1944). So far, only one C_1 progeny of a ligneous plant seems to have been reported in the literature. DERMEN (1947) obtained 12 triploid plants from the $C_0 \times$ diploid cross in pear on flowering two years after the treatment. In the following paper the result of an eight-year colchicine experiment with *Alnus glutinosa* is presented.

C_0 GENERATION.

The material. — The treatment, which was undertaken in 1941 and carried out by C. EKLUNDH, proceeded by placing dormant seeds on filter paper moistened with 0,2 % colchicine solution in Petri dishes. Germination soon commenced and the seedlings were left in the col-

chicine solution as long as they exhibited any development, about three weeks. After that they were transferred to soil. Owing to the long treatment the mortality was high. About twenty plants survived the first two years of cultivation in the nursery. These were subsequently further cultivated in the experimental garden. Half the number of trees were here allowed to develop undisturbed, while half were submitted to interferences of various kinds with a view to stimulating flowering. In the spring of 1948, seven years after the treatment, one tree flowered for the first time and was then crossed to an untreated diploid of similar origin. In the autumn of 1949 this tree and another four have developed flower-buds. The following account is confined to these five trees. Four of them have grown without interference since the treatment and have accordingly flowered spontaneously, one has been subjected to root pruning, girdling, and trimming of the crown. Hence the flowering of this tree may have been elicited by the measures taken.

Morphological features. — The young seedlings had the appearance characteristic of colchicine effect, having greatly thickened cotyledons and a clavately swollen radicle. They were much stunted in growth. When the foliage leaves appeared, they presented distinct anomalies, often being very asymmetric, irregularly lobate and with abnormally deep teeth. The leaves kept this appearance throughout the years (Fig. 1). Different trees differ greatly in their leaf morphology, but within trees the leaves are of a uniform type. No vegetative segregation with appearance of normal branches in otherwise abnormal crowns has been observed. The now eight year old trees, which have grown without interference, are about four metres tall, *i. e.* two—three metres shorter than normally. The shape of the trees is distinctly irregular, with more or less crooked stems and asymmetric crowns.

The length of the stomata was measured the year of treatment and after that in three different years. In 1945 the length of the wood-cells in the coarser branches was also measured. These dimensions as well as the chromosome numbers in the pollen mother-cells are recorded in Table 1. As controls ten trees of the same origin as the C material have been used and the highest and lowest figures for these trees are given in the table. On each measuring occasion 50 stomata from each of three leaves from each tree were measured. Hence the mean values have reference to 150 measurements. The measurements were carried out with objective $\times 40$ and ocular $\times 15$, one unit being $= 2{,}4\ \mu$. For measurement of the wood-cells the wood was macerated by boiling with alcoholic nitric acid for two hours according to KÜRSCHNER's technique.

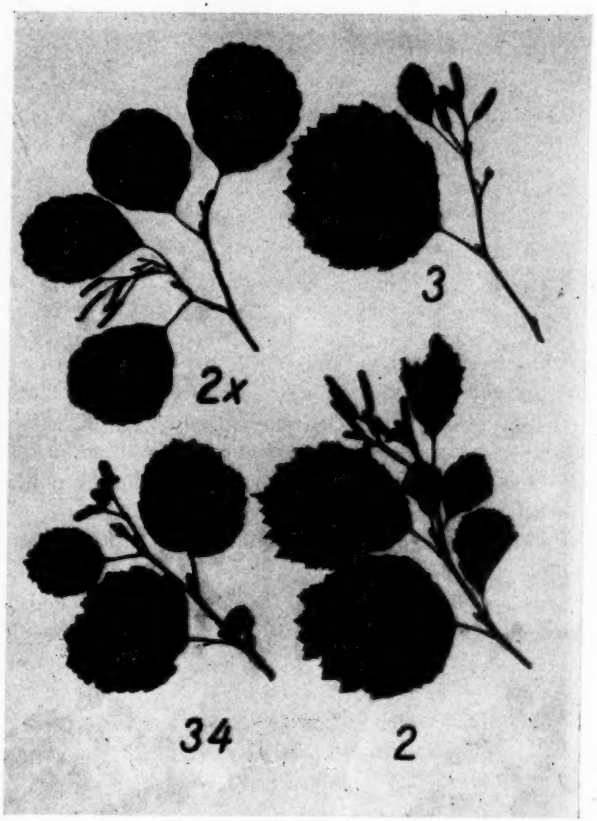


Fig. 1. Branch-tips with leaves and buds. $2x$ = diploid control. 2, 3 and 34 C_0 trees.

The wood-cells were thereupon diluted in water, and glycerine was added when the preparations were put in order. The preparations were projected with a microscope, objective × 5 and ocular × 7, the length of the cells being then determined with dividers and a micrometric scale on the surface of projection. The mean figures have reference to 100 cells, and one unit equals 0,01205 mm.

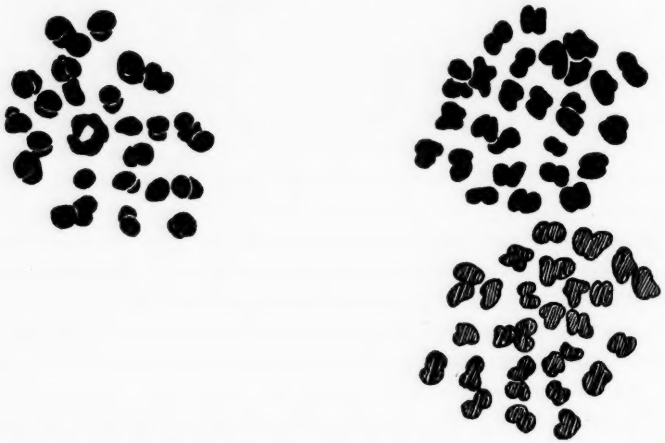
From the table it is seen that the relations between the stomata of the five C_0 trees as well as between the C material and the controls have been fairly constant during the eight years. Three of the C_0 trees have significantly longer stomata than the controls while two are

TABLE 1. *Length of stomata and wood-cells of the C_0 generation.*

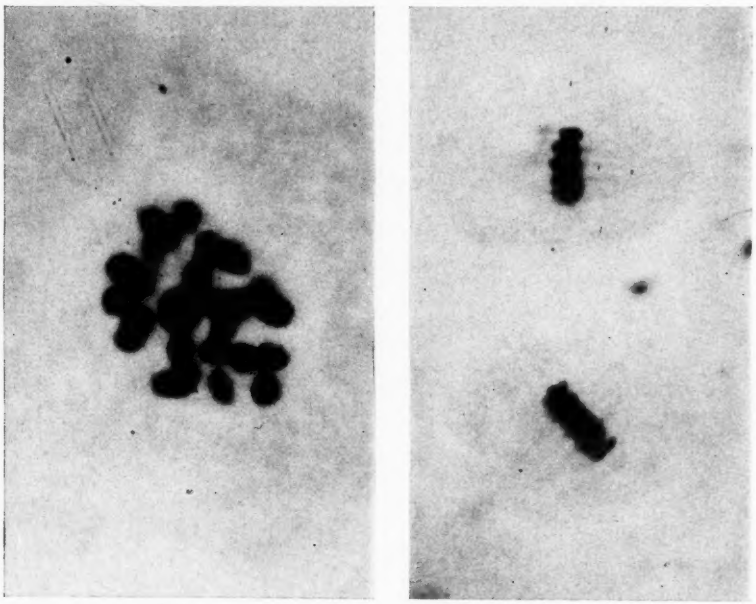
Tree No.	Length of stomata				Length of wood-cells	Meiosis
	1941	1943	1945	1949	1945	
2	16,75 ± 0,17	14,25 ± 0,16	15,98 ± 0,15	15,32 ± 0,17	44,13 ± 0,83	2*x*
3	17,99 ± 0,15	13,57 ± 0,15	15,93 ± 0,14	14,99 ± 0,16	47,59 ± 0,92	4*x*
4	17,44 ± 0,13	14,47 ± 0,15	14,63 ± 0,14	17,64 ± 0,16	—	4*x*
31	13,08 ± 0,10	11,83 ± 0,13	13,10 ± 0,15	12,43 ± 0,12	45,82 ± 0,92	4*x*
34	12,48 ± 0,19	12,61 ± 0,19	13,43 ± 0,15	12,82 ± 0,14	49,87 ± 0,92	4*x*
Control						
maximum	—	—	14,30 ± 0,17	14,67 ± 0,17	43,19 ± 0,81	—
minimum	—	—	12,99 ± 0,14	13,33 ± 0,14	32,47 ± 0,53	—

entirely within the diploid variation. All the four C_0 trees whose cells were measured have higher values than the control material. The P-M-C's are tetraploid in four trees and diploid in one. These figures clearly illustrate the mixoploid nature of the C_0 individuals. Tree No. 2 has tetraploid stomata, tetraploid wood-cells but diploid pollen mother-cells. Trees Nos. 3 and 4 are in all respects tetraploid, trees Nos. 31 and 34 have diploid stomata, tetraploid wood-cells and tetraploid pollen mother-cells.

Male meiosis and fertility. — As already mentioned, tetraploid P-M-C's have been observed in four of the C_0 trees and diploid P-M-C's



Figs. 2—3. I-M and I-A in a C_0 tree with tetraploid archespore. — 2 = I-M in polar view with probably $6_{IV} + 16_{II}$. — 3 = I-A in polar view with the chromosome distribution 28—28 (× 5200).



Figs. 4—5. Photomicrographs of I-M. — 4 = same cell as in Fig. 2 (× 5200). — 5 = I-M in side view (× 4000).

in one. The observations are based on an ample material from several catkins belonging to each tree. In all cases the chromosome numbers of the pollen mother-cells have been uniform. By this it is, of course, not meant that this uniformity is absolute. For instance, it is conceivable that tree No. 2, in which only diploid P-M-C's were found, may have had tetraploid archespores in catkins on other branches than those studied.

The general course of meiosis is characterized by a varying number of quadrivalents and a corresponding number of bivalents in I-M. Univalents have not been observed. Fig. 2 shows a I-M seen from above, and in Fig. 4 a photomicrograph of the same cell is reproduced. The syndesis in this cell is probably $6_{IV} + 16_{II}$. It is impossible to analyse the I-M plates with absolute exactness, for two bivalents lying close together can sometimes not be distinguished from a quadrivalent. For 12 P-M-C's that could be analysed with satisfactory security the following quadrivalent frequency was obtained.

Number of quadrivalents	3	—	5	—	—	8	9	—	11	12	13
» » P-M-C's	1		2			1	3		3	1	1

Viewed from the side the I-M plates give a complete regular impression (Fig. 5). There is no possibility of closely studying the shape of the quadrivalents. The separation in I-A is very regular, 28—28, and the chromosome number can be readily determined at this stage (Fig. 3).

TABLE 2. *Diameters of the pollen grains in C_0 and controls.*

Plant No.	Diameters of pollen grains										
	10	11	12	13	14	15	16	17	18	19	Mean
34 (C_0)				3	11	32	54	37	11	2	$16{,}01 \pm 0{,}09$
9 ($2x$)	1	21	36	23	13						$12{,}28 \pm 0{,}11$
129 ($2x$)		20	64	57	14						$12{,}42 \pm 0{,}08$

TABLE 3. *Number of pores in the pollen grains of C_0 and controls.*

Plant No.	Number of pores						
	2	3	4	5	6	7	Mean
34 (C_0)	1		3	46	52	2	$5{,}48 \pm 0{,}08$
9 ($2x$)	1	3	39	9			$4{,}08 \pm 0{,}08$
129 ($2x$)	1		84	61	1		$4{,}41 \pm 0{,}06$

The pollen quality could be studied only in the first flowering tree, No. 34. The pollen was on an average larger than normally, as is evident from Table 2, and somewhat more varying than in the control trees, Nos. 9 and 129. There was a slight frequency of aborted grains. Morphologically, too, the C_0 pollen differed from the normal in that the number of pores was larger, $5{,}48 \pm 0{,}08$ compared with respectively $4{,}08 \pm 0{,}08$ and $4{,}41 \pm 0{,}06$ for the two control trees (Table 3).

Female fertility. — When tree No. 34 flowered in the spring of 1948, it was used as mother in a cross with a diploid tree of the same origin. About 140 ripe catkins could be harvested and these gave 21,14 gm of seed. The 1000-grain weight was 2,42 gm compared with 0,73—1,44 gm for the controls. High seed weight seems to be a characteristic quality of autotetraploids. For instance, this was the case in barley (MÜNTZING, 1943), flax (LEVAN, 1942) and in a number of other plants (HOWARD, 1947). The germinability of the seed on Jacobsen's apparatus was 41 %, which is a fairly normal value for *Alnus glutinosa*.

The C_1 generation. — Seed obtained from the above-mentioned $C_0 \times$ diploid cross was sown in the greenhouse in March, 1949. The seedlings were transplanted once in the greenhouse and were planted out into frames in May, where the material was cultivated during the

summer. The C_1 progeny was cultivated on fairly large plots, altogether 1904 plants being planted. Between these, smaller control plots of a closely related diploid cross were inserted. The number of control plants was 672. Of the 1904 C_1 plants there remained 1898 in the autumn, and of the 672 control plants, 668, *i. e.* practically speaking no loss.

Growth. — During the summer the C_1 progeny have been characterized by a particularly robust growth and possessed strikingly large leaves. Table 4 gives the plant heights after the end of the growing period for the C_1 progeny ($3x$) and the diploid control ($2x$). The mean height of the control is $40{,}6 \pm 1{,}2$ cm and of the C_1 progeny it is 60 cm. No calculation has been made of the standard error for the C_1 progeny because the latter's variation is far from normal, as is to be seen from Table 4 and still clearer from the diagram in Fig. 6. The variation of the diploid control is practically normal, while the curve for the C_1 progeny is strikingly skew with a suggestion of bimodality. If the maximum of the curve at 80 cm is taken as the starting-point, the skewness appears as caused by a powerful overrepresentation of minus-variants. The most plausible interpretation of such a variation in a progeny from a tetraploid × diploid cross would be to assume a higher frequency of numerical aberrants originating from an irregular course of meiosis in the tetraploid. The chromosome numbers have been determined in 14 plants taken at random from the C_1 progeny. All had exactly the triploid number, *i. e.* $2n = 42$ (Fig. 7). Hence the frequency of numerical aberrants, if such at all appear, must be very low and cannot account for the skewness of the variation curve. Unbalanced chromosome constitutions can, however, also occur among numerical triploids, *e. g.* individuals that are tetrasomic for one chromosome and disomic for another. Moreover, it is not necessary to seek the cause of the skewness in aberrants or in lacking chromosome balance; it can also be thought to be caused by there being a different gene-interaction in triploid from that in diploids. Nor it is necessary to describe the skewness as overrepresentation of minus-variants. If a normal curve is

TABLE 4. *Plant lengths of the triploid C_1 generation and of the diploid control.*

Progeny	Plant length in cm																			Number of plants	Mean cm
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110		
$2x$		1	13	40	47	58	75	74	85	78	57	62	44	16	9									668	$40{,}6 \pm 1{,}2$
$3x$		11	22	43	61	73	73	62	83	69	93	88	116	156	168	176	197	165	141	63	28	6	4	1898	(60,0)

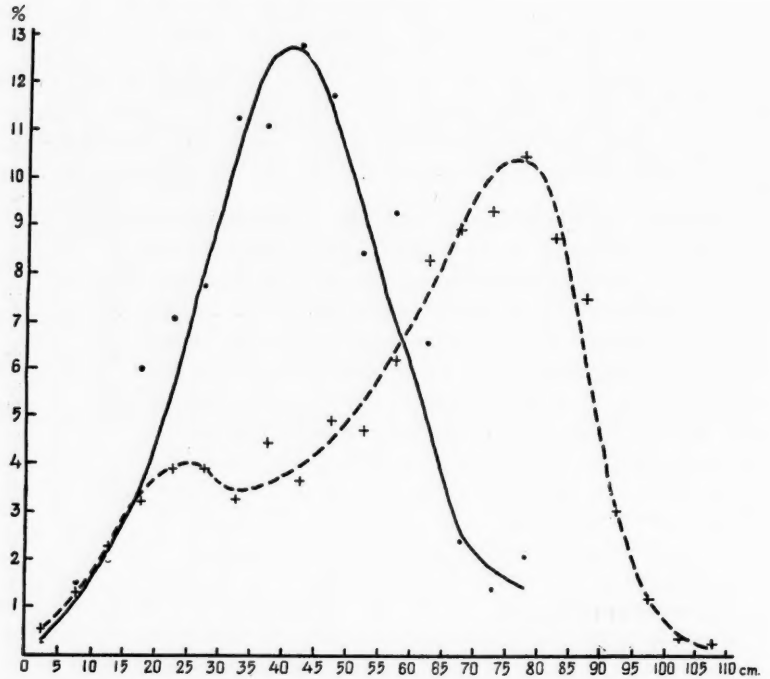


Fig. 6. Diagram showing the variation in plant height of the triploid C_1 progeny (— — —, +) and the diploid control (———, .).

constructed of the range of variation shown by the $3x$ progeny the skewness appears, instead, as an underrepresentation of minus-variants and an overrepresentation of plus-variants. In any case the skewness will disappear in course of time if the material is cultivated further conformably with forestry practice.

At the planting in the forest of the yearling plants the 10—20 per cent weakest will be rejected, which means that, already then, the minus portion of the variation curve will be cut off at about 30 cm. Should there later exist a strong correlation between the first year's growth and subsequent development, the frequency of minus-variants will be reduced at every thinning of the stand and the curve of variation will approach normality more and more closely.

Examination of the curves of variation in Fig. 7 reveals another differences between the triploid and the diploid variation. The range of

variation of the triploid extends over 22 class-breadths while that of the diploid lies within 16, *i. e.*, expressed in class-breadths, the variation range of the triploid is 37,5 % wider than that of the diploid. This condition will be discussed below at somewhat greater length.

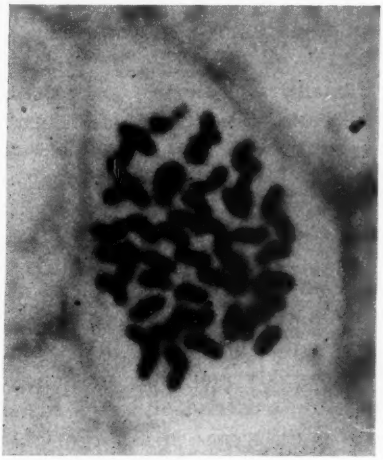


Fig. 7. Photomicrograph of a cell from the root meristem of a C_1 plant with $2n = 42$ ($\times$ 3900).

Stomata.—The stomatal length has been determined for ten plants taken at random from the triploid as well as the diploid population. For the triploids the total mean obtained was $15{,}_{69} \pm 0{,}_{15}$ units with an individual variation from $14{,}_{53} \pm \pm 0{,}_{12}$ to $15{,}_{93} \pm 0{,}_{12}$. That of the diploids was $13{,}_{24} \pm 0{,}_{12}$ with an individual variation from $12{,}_{79} \pm \pm 0{,}_{11}$ to $13{,}_{89} \pm 0{,}_{15}$. The length of the stomata in the triploids therefore exceeds that of the diploids by 14 % on an average and the two variations are quite distinct from each other.

Wood properties. — SCHLÖSSER (1937), SCHWANITZ (1949) and many other authors between them have observed that the water content, especially of the leaves, is higher in autopolyploids than in their original diploid forms. For this reason the water content has been determined in the stems of the diploid and triploid populations under present discussion. At the same time the specific weight of the wood in a moist and in a dry state as well as the shrinkage on drying has been computed. The results are summarized in Table 5.

Sixteen plants taken at random from each chromosome race were used for the determinations. In the table the water content has been commuted into dry-substance content. This content was determined in samples taken from the middle of the plants, while the specific weights and the shrinkage were worked out from specimens at the base of the plants. The 16 triploid plants used weighed in the moist state 55 % more than the diploid and in a dry state 47 % more. In spite of the magnitude of the differences these are barely significant. This is due to the great dispersion of the material and the relatively small number of determinations. Had a larger material been examined, the differences would

TABLE 5. *Some properties of the wood of diploid and triploid alder plants.*

	Weight per plant gm		Per cent	Specific weight		Shrinkage
	fresh	dry	dry matter	fresh	dry	per cent
$2x$	11,73 ± 1,50	5,84 ± 0,76	49,50 ± 0,49	0,786 ± 0,005	0,497 ± 0,007	21,2 ± 0,69
$3x$	18,17 ± 2,34	8,61 ± 1,14	46,86 ± 0,85	0,793 ± 0,011	0,466 ± 0,008	20,1 ± 0,58
$3x/2x$. 100	155	147	95	101	94	95
t	2,320	2,013	3,620	0,583	3,028	1,225
p	0,05 — 0,02	0,05	< 0,01	0,6 — 0,5	< 0,01	0,3 — 0,2

undoubtedly have been at least as large as now, while the mean error would have been reduced. The conclusion may therefore be safely drawn that during the first summer the triploid plants had produced considerably more dry substance than the diploid. The fact that the dry-substance content is 5 % higher in the diploids does not affect this, as the difference in moist weight has so high a value. No difference appears in the specific weight of the moist wood, but in the dry state the diploid wood is significantly heavier, with a difference of 6 %. The difference in wood shrinkage on drying is not significant. From the figures reported the pore volume of the wood can be estimated (HÄGGLUND, 1939; JOHNSSON, 1945). For the triploids it is found that 70,13 % of the dry wood volume consists of air spaces while the corresponding value for the diploids is 68,14 %. Here, the larger elements in the triploid wood make themselves felt.

It would be premature to draw any conclusions concerning the technical quality of the mature material from the investigations that can be made on the one-year old wood. Possibly a tendency to a somewhat lighter and looser material may be suspected in the one-year old wood of the triploids.

DISCUSSION.

The mixoploids produced by colchicine treatment seem in their main features to be periclinal chimæras with fairly uniform tissues. The epidermis may be diploid with stomata of normal size and the underlying tissues tetraploid as in the C_0 trees Nos. 31 and 34. In C_0 tree No. 2 more complicated conditions seem to be present. In all probability the epidermis is here tetraploid, and so are the wood-cells, while the intervening layer of cells, which gives rise to the anthers, must be diploid. Similar periclinal chimæras have been observed to follow colchicine treatment in *Datura* (SATINA and BLAKESLEE, 1941) and in *Vaccinium*

(DERMEN and BAIN, 1944). These authors have shown that the growing points in the widely separated genera *Datura* and *Vaccinium* are built up of three germ-layers. Presumably this condition is general among the angiosperms and also prevalent in *Alnus*. The different meristematic strata appear to react fairly independently of each other, whereas the cells in the same layer seem to respond simultaneously to the influence of colchicine. Periclinal chimæras arising are evidently stable, at any rate they have lasted eight years in the present material of *Alnus glutinosa*. Without doubt it not unfrequently happens that the chromosome number in the meristematic cell-layer of the growing-point are doubled in all or the majority of cells. C_0 tree No. 3 seems to be an instance of this. Undoubtedly there also arise sectorial chimæras and a vegetative segregation can also probably originate in periclinal chimæras if adventitious buds can be formed in different germ-layers and continue to develop.

In any case, as in herbs, polyploid races can be raised in the C_1 of ligneous plants in spite of the long time that elapses between treatment of the C_0 material and the flowering. This period was seven years in the case of the material of *Alnus glutinosa* treated at Ekebo. The utility of the colchicine method for the improvement of forest trees, fruit trees (DERMEN, 1947) and bacciferous bushes (VAARAMA, 1947) is therefore definitely established.

The triploid C_1 progeny of *A. glutinosa* possess an essentially wider range of variation than the diploid control. The question is whether we have here a special case or whether autopolyploids generally possess a wider variation than their diploid prototypes. This problem seems to have received but little attention. FABERGÉ (1936) demonstrated, however, that the variation in fruit weight of tetraploid tomatoes is narrower than that of the diploid. One source of increased variation in autopolyploids is, of course, to be sought in disturbances of meiosis, which may lead to numerical aberrants and incomplete genomes in euploids. However, the possibility has to be considered that the effect of such disturbances can already be eliminated at the gametic stage. Another source of increased variation in polyploids may be the increased number of loci and the consequently greater possibilities of recombination afforded. If the character investigated is oliogenously conditioned and extreme factorial combinations are consequently relatively common, an increased variation is doubtless also to be expected in the polyploid. In polygenic systems with a low probability for rather extreme factorial combinations

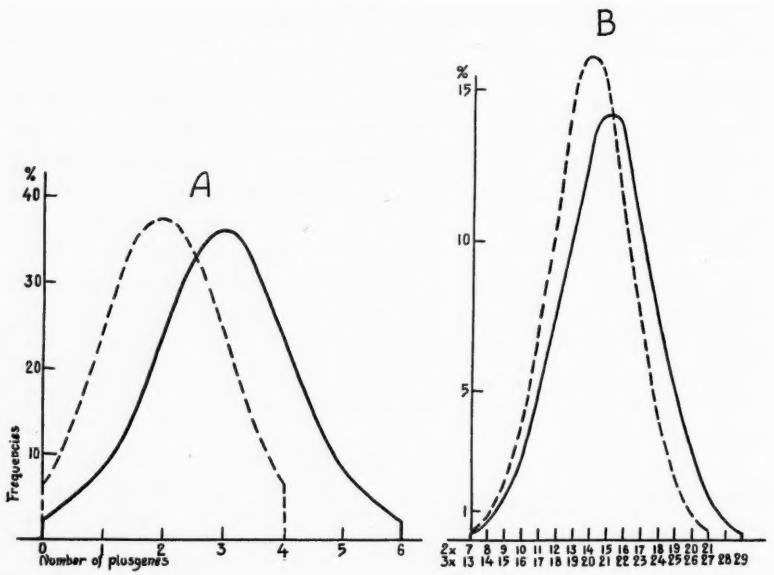


Fig. 8. Variation curves for gene frequencies in diploid (— — —) and corresponding autotriploid (———) populations. A with two heterozygous unlinked genes. B with 14 unlinked genes. The segregation in the tetraploid parent to the triploid population is assumed to be bound up with a random distribution of the four chromosomes.

a widening of the variation range is not so self-evident. This view is illustrated by the curves in Fig. 8.

The curves are constructed on the assumption that the variation shown by the diploid in case A is conditioned by two unlinked gene-pairs and in case B by 14 unlinked gene-pairs ($x = 14$ in *Alnus*). It is further assumed that the diploid variation in A represents progeny from a cross between two double heterozygotes and in B from a cross between two individuals that are both heterozygous for all 14 factors concerned. It is presumed that the chromosome numbers of these heterozygotes are doubled and that the tetraploids are back-crossed to the diploid heterozygotes. The segregating mechanism of the tetraploids is supposed to consist in a random distribution of four loci. If all the pairs of factors are denoted + and —, the frequencies are obtained for individuals with different numbers of + and — factors for diploids from $(++ : 2+- : --)^n$ and for triploids from $(++ : 4+- : --)^n$ $(+ : -)^n$. Hence the curves in A represent the frequencies obtained on expansion of these

expressions for $n = 2$ and in B for $n = 14$. The surface of the curves is the same in A and B, and the percentage frequencies have been given. In case A all possible combinations are readily realized even in a small experimental material. The range of variation for the triploid population is 50 % wider than for the diploid. In case B, on the other hand, it cannot be expected that all possible combinations will occur even in the diploid population in an experimental material of normal extent. Individuals with fewer than seven or more than 21 plus-factors will practically speaking be entirely absent.

In the triploid population still larger portions of the two wings of the variation curve will be cut off as individuals with fewer than 13 and more than 29 plus-factors do not occur in practical frequencies. The variation range of the triploid will be only 11 % wider than that of the diploid, and in a rather limited material it is not certain that the variation realized is greater in the triploid than in the diploid. The higher the number of factors involved the lower will be the difference between the two populations. These curves have reference to the genotypic variation. How faithfully the observable phenotypic variation follows the genotypic frequencies will naturally depend upon manifold circumstances. If the plus-factors have a purely cumulative and additive effect, the minus-factors are without any effect of their own, and if no complications on account of interactions occur, the phenotype curves will follow the genotype curve. If the additive effect ceases or diminishes when the number of plus-factors have reached a certain limit, this will in the first place influence the variation of the polyploid, which will then decrease. On the other hand, interaction of factors can increase the variation of the polyploid.

The positions of the diploid and polyploid curves in relation to each other depend, of course, upon other conditions, such as chromosome-number optimum. The author's opinion is that there is no reason to assume a larger primary variation in autopolyploids in general than in diploids. When such makes its appearance, as in the colchicine-treated alder material, it seems to be most probable that a small number of »large genes» are present, unless the increased variation can be attributed to chromosomal causes. It is quite a different matter that repeated selection, artificial or natural, ought to be able to lead to more extreme forms in polyploids than in diploids on account of the increased possibility of successive accumulation of certain genes that follow upon the increased number of loci.

SUMMARY.

(1) C_0 trees derived from germinating seeds of *Alnus glutinosa* that had been colchicine-treated in 1941 have been examined in connection with the flowering in 1948 and the development of flower-buds in 1949. It has been found that in essentials the trees are periclinal chimæras with diploid or tetraploid epidermis and diploid or tetraploid internal tissues. The trees have retained their chromosomal constitution during the eight years the investigation has been in progress. Four of the five trees studied produce diploid gametes. Their meiosis is normal for autotetraploids with a varying number of quadrivalents.

(2) A one-year-old C_1 offspring from a $C_0 \times$ diploid cross has been described. This is characterized by powerful growth and large leaves. The curve of variation for the population is skew and the range of variation is wider than in the diploid control. The dry-matter content of the wood is lower in the triploid than in the control and the specific weight of the wood in a dry state is lower.

(3) The variation range of autopolyploids in comparison with their diploid prototypes has been discussed.

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THE MUTAGENIC EFFECT OF RADIO-PHOSPHORUS IN BARLEY

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IN 1949 EHRENBERG, GUSTAFSSON, LEVAN and VON WETTSTEIN studied the seedling lethality and chromosome disturbances caused by radio-phosphorus (P32) in seeds of barley and wheat and in roots of *Allium cepa*. On the basis of their results they predicted that P32 must have a very high mutagenic effect. The cytological disturbances after treatments with 5—10 μC/seed were extremely frequent, corresponding to effects of 25.000 *r*-units or more. Such a mutagenic effect has recently been demonstrated by GILES and LEDERBERG (1948) for *Neurospora* and by BATEMAN and SINCLAIR (1950) for *Drosophila*. The present work, which is here given as a preliminary report, confirms this statement with barley as the test material.

In the spring of 1949, barley seeds of the sieve fraction 2,50—2,75 mm. were treated with P32 according to the method described by EHRENBERG *et al.* (1949). The strain used was a line-selection (01518 b_{10}) out of the high-yielding Bonus barley. P32 was supplied as phosphate ions (from the Atomic Energy Commission, U. S. A.) by soaking the seeds for 96 hours in petri dishes (14 cm. diam.) with the calculated amounts of 0,625, 1,25, 2,50, 5,00, 7,50, and 10,00 μC of P32 and 0,1 cc. of distilled water per seed.The germination proceeded at room temperature. Immediately after the treatment the germinated seeds were sown out in the field. Five random seeds per dose were analysed in order to determine the absorbed amount of P32 (*cf.* EHRENBERG *et al.*, 1949, p. 475).

The gathered data from this field experiment are presented in Table 1. The rates of mutation in the second generation have been based on chlorophyll mutations, determined at the seedling stage under greenhouse conditions.

Simultaneously, experiments were carried out involving dry and presoaked seed material irradiated with X-rays and fast neutrons, thus permitting direct comparisons with the P32-series. On a later occasion the complete data of the X-ray and neutron experiments will be

published, together with similar investigations carried out in previous years.

In 1948 GUSTAFSSON and MAC KEY compared the actions of X-rays and neutrons on dormant seeds in barley. They found the following conditions prevailing.

An increasing dosage of X-rays causes a very high mortality of seeds and seedlings. When doses of 20.000 or 25.000 *r*-units are applied, just a few per cent of the plants survive and are able to produce spikes and seeds. The resulting plants, it is true, show a conspicuous decrease in fertility, but this rarely falls below 50 or 60 per cent. Furthermore, the mutation rate of the X_2 generation declines with a rise in X-ray dosage (*cf.* also FREISLEBEN and LEIN, 1943; GUSTAFSSON, 1947; GUSTAFSSON and NYBOM, 1949). Fast neutrons act differently. Even after high doses, 15.000 disintegrations [1] (per gram Al and minute) or still more, the number of surviving plants is nearly normal. The plants are, however, severely affected due to the accumulation of gross chromosome abnormalities, and the fertility falls as low as 10—20 per cent. The absolute mutation rates are decidedly higher than after X-rays; the rare and drastic mutations are more frequent and there is a certain approach to a linear relationship between dose and number of induced mutations.

Presoaked seeds react in an entirely different way. From earlier examinations (GUSTAFSSON, 1947, and here cited literature) it is known that seeds soaked in water before X-raying show an inferior sprouting ability, a higher number of dead seedlings, a higher X_1 sterility and a higher mutation rate than dry seeds. In the 1949 year experiment these effects were verified except for the mutation rate which was inferior to that of irradiated dry seeds. Presoaked seeds partially react to neutrons in the same way as to X-rays. The seedling mortality is very high, the number of surviving plants decreasing from 80 to 4 per cent with the highest dose (circa 14.000 disintegrations). The fertility of the badly affected surviving plants approaches zero. The mutation rates per plant and spike progeny reach very high absolute values.

The declining curve of mutation rates in X-irradiation depends on two factors: a high mortality of the treated seeds and seedlings and a pronounced elimination of badly affected cells or cell groups within the germinating seeds. This elimination is more pronounced with presoaked

[1] In this connection we must correct the neutron doses given by GUSTAFSSON and MAC KEY in their paper of 1948. The doses were in fact calculated sixteen times too high. A more accurate technique worked out by Dr. A. HEDGRAN of the Nobel Institute of Physics, Stockholm, now permits more definite measurements.

TABLE 1. *The effect in the 1st and 2nd generation after treating barley seeds (01518 b_{10}) with radiophosphorus.*

Field No.	Dose	Amount of P32 absorbed, µC/seed	Analysis of plant survival in 1st gen.			Mean plant fertility %	Analysis of mutation frequency in 2nd generation					
			No. of grains sown	Percentage of			No. of plant progenies examined	No. of spike progenies examined	No. of chlorophyll mutations	Mutation rates in % per		
				seedlings	mature plants					spike progeny	spike progeny and µC based on the amount of P32	
											supplied	absorbed
1949—24—42	Control	—	150	96,0	90,0	95,6 ± 0,38	98	218	0	0,0	—	—
» —36	0,625 µC	0,30	300	90,7	81,3	95,1 ± 0,42	99	271	5	1,8	3,0	6,2
» —37	1,25 »	0,55	300	91,3	85,0	88,7 ± 0,90	100	263	7	2,7	2,1	4,8
» —38	2,50 »	0,75	300	83,7	73,3	83,7 ± 1,00	97	243	13	5,3	2,1	7,1
» —39	5,00 »	1,05	450	79,3	46,7	70,2 ± 1,55	94	252	12	4,8	1,0	4,5
» —40	7,50 »	1,50	450	68,4	28,2	61,1 ± 1,56	95	262	16	6,1	0,8	4,1
» —41	10,00 »	2,00	450	41,1	6,7	53,2 ± 2,47	26	78	5	6,4	0,6	3,2

than with dry seeds. With neutron treatments there is a similar elimination going on with regard to sex-cells, giving rise to an inferior seed setting. Consequently, there are factors restricting the realization and number of gene mutations both with X-rays and neutrons. Radiophosphorus, which for its mode of action ought to be compared with presoaked rather than with dry seed series, holds an intermediate position between X-rays and neutrons. It causes a better plant survival than these two agents. The resulting plant fertility, on the other hand, is lower than with X-rays but higher than with neutrons. With regard to the mutation rates, it holds a similar intermediate position, as visualized by the following survey:

	Neutrons Mean of five doses (1400—13900 dis.)	Radiophosphorus Mean of six doses (0,3—2,0 absorbed microcuries per seed)	X-rays Mean of four doses (5000—20000 *r*)
Calculated per plant progeny	21,3 %	12,4 %	10,4 %
» » spike »	8,0 »	4,5 »	2,9 »

The mutation range obtained with radiophosphorus and neutrons is wider than with X-rays.

X-rays and phosphorus 32 both act through electrons. Hence it may be interesting to compare the action of equal energy quantities of both types of electrons. One microcurie of P32 absorbed corresponds to an energy taken up by the plant amounting to $(0{,}15 - 0{,}50) \cdot 10^6$ ergs/gram of tissue, *i. e.* 3600 ± 2000 *r* (the maximum error is given). In the present experiment 19.500 *r*-units were required in order to assure the same average mutation rate as 1 μC of P32. Consequently, we state that P32 is more effective than are X-rays, probably because P32 is localized to certain cells or cell groups, whereas the X-ray energy is scattered equally to all cells of the irradiated embryo.

To sum up: Radiophosphorus is highly mutagenic. In its mode of action it lies between X-rays and neutrons. The mutation range, obtained with radiophosphorus, is wider than with X-rays. P32 is easy to handle and to apply. Hence it is expected to become very useful in mutation work and plant breeding, especially when dealing with species like trees and shrubs which are largely propagated by vegetative means.

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ABSTRACTS

PETER BERNSTRÖM: Does the fertility of intraspecific hybrids in autogamous species improve in consequence of a heterotic effect?

As a part of an investigation of the relationships between the North-European *Lamium* species (BERNSTRÖM, 1941, 1944, and unpublished), intraspecific hybrids in *L. purpureum* and *L. hybridum, i. a.*, have been studied for some years, with the main purpose of examining whether some of the hybrids would turn out to be partially sterile. During these studies the hybrids in *purpureum* have proved to be generally characterized by manifest heterosis, shown by measurements of several characteristics (height of the stem, length of the branches, number of internodes, degree of leaf-indentation, and time of maturity); an account of these investigations will appear later on. On the other hand, it has not been possible to prove any general heterosis in the hybrids within *hybridum*. Studies on the degree of seed-setting have revealed that in *purpureum* this is not lower in the hybrids than in the pure lines; on the contrary, the seed-setting is better on an average in the hybrids, and the same tendency does exist in *hybridum*, too.

Both *purpureum* and *hybridum* are autogamous to a very high degree, and thus consist of pure lines, in many cases clearly differing in appearance. The fertility of the lines is very good, as is to be expected; the percentage of apparently good pollen grains is on an average 97—99 in both species. The seed-setting is also good, but it is better in the diploid *purpureum* ($2n = 18$) than in the tetraploid *hybridum* which is an amphidiploid species (BERNSTRÖM, unpubl.). The degree of seed-setting has been determined on the 2 (—3) whorls situated at the lowest flowering nodes of the stem. At least 20 calyces and, as a rule, still more have been examined; they have been studied after the dropping of the ripe seeds, it then being possible to count the aborted ovules *in situ*. The estimation of the degree of seed fertility has been based on the fact that the gynoecia always consist of four ovules; below the seed fertility is expressed in percentage of ripe seeds.

For the experiments with *purpureum* 10 lines have been used, one of which (called p26) originates from Switzerland and another one (p85) from Syria, the other ones being collected in South Sweden. The results of the studies on the seed-setting in *purpureum* are summarized in Table 1. As can be seen from this, two generations a year have been cultivated in 1944 and 1945, the first one starting in the second week of April and the second one starting in the last week of July.

By »relative value» is meant the mean value of the F_1 hybrids expressed in percentage of the average of the two mean values in the parent lines. The number of plants examined is indicated by n. It is evident from the table that the relative values almost throughout exceed 100, 18 being > 100, 2 values $= 100$ and only one < 100. χ^2 for the distribution 18 : 1 instead of the distribution 9,5 : 9,5, theoretically expected, will be 15,21 ($P < 0{,}001$); if the relative values of 100 and < 100 together are contrasted with the others, the distribution being 18 : 3, χ^2 will be 10,71 (P very nearly approaching 0,001) and,

TABLE 1. *Seed-setting in pure lines and crosses between pure lines of Lamium purpureum.*

Generation (year)	1944 — 1			1944 — 2			1945 — 1			1945 — 2			1946			1949		
Parent lines	$\bar{x}$ value	rel. value	*n*	$\bar{x}$ value	rel. value	*n*	$\bar{x}$ value	rel. value	*n*	$\bar{x}$ value	rel. value	*n*	$\bar{x}$ value	rel. value	*n*	$\bar{x}$ value	rel. value	*n*
Pure lines																		
p1	95,7		13	92,9		9	94,8		14									
p2	93,7		14	95,6		9	95,4		14							94,6		7
p25				94,2		11	89,0		14				90,4		3			
p26	82,4		13	82,2		8	90,8		16	90,3		8	79,6		4			
p29	90,1		14	93,3		11	93,3		14	88,8		11						
p31				did never ripen			95,3		14									
p51													87,9		5			
p53										93,1		11	98,8		5			
p54													96,5		5			
p85																88,0		7
F_1 hybrids																		
p1 × p2	92,9	98	27															
p1 × p26	91,9	103	21	90,5	103	14												
p1 × p29	93,9	101	10	93,2	100	14												
p1 × p31				94,5	—	13	98,5	104	2									
p2 × p25				95,5	101	14												
p2 × p26				90,9	102	14	93.4	100	14									
p2 × p85																96,9	106	7
p25 × p26				91,6	104	14	95,3	106	12									
p25 × p51													95,4	107	7			
p26 × p29	87,7	102	6	90,4	103	12												
p26 × p53										93,1	102	12						
p26 × p54													94,6	107	3			
p29 × p31							96,7	103	9									
p29 × p53										95,5	105	9						
p51 × p53													97,5	104	7			
p51 × p54													98,6	107	3			

thus, this distribution proves to be highly significant, too. Certainly, the seed-setting of the hybrids exceeds that of the better parent line in 7 cases only, the remaining hybrids mostly having an average value but a little exceeding the common one of the both parent lines; in other words, the relative values exceed 100 by very little. An exceptionally low degree of seed-setting especially in the line p26, which is a parent component in a good half of the hybrids, may contribute to the excess in seed-setting of the hybrids. If only the hybrids without p26 are included, however, the following distribution of the relative values will be obtained: $8 > 100$, $1 = 100$ and $1 < 100$. The distribution 8 : 1 instead of the theoretically expected 4,5 : 4,5 corresponds to a

$P = 0{,}0195$; if the two hybrids are combined, the relative values of which are $= 100$ and < 100, the distribution 2 : 8 instead of 5 : 5 thus obtained will correspond to a $P = 0{,}0547$. Thus, the predominance of the hybrids is still strong, irrespective of the distribution of the relative values.

As none of the pure lines nor the hybrids have been cultivated in all the plant generations, no hybrid is actually being studied in more than two generations at the most. Thus it would not be quite correct to compare directly the average value of all the pure lines with the corresponding value of the hybrids, as such a comparison is likely to be influenced both by differences between years and generations and by genotypical differences between the lines. However, in order to get an idea of the tendency of the difference between parent lines and their hybrids, the average values indicated above have been calculated; they are 91,5 for pure lines (the mean value of the hybrids p1 × p31 in 1944 — 2 is not included) and 94,0 for the hybrids.

For most of the hybrids dealt with above, the percentages of apparently good pollen grains have been determined, the relative values of which being calculated in the same way as for seed-setting; and it is worth mentioning that they are distributed fairly evenly round the number 100. The figures are as follows: $3 > 100$, $10 = 100$ and $6 < 100$.

In the same way as for *purpureum*, the seed-setting has been examined in 13 different hybrids in *hybridum*, which have been cultivated in 5 plant-generations. The hybrids are composed of 10 pure lines. The seed-setting of the hybrids as compared to that of their parent lines predominantly exceeds the relative value 100. The distribution of the relative values is: $10 > 100$, $0 = 100$, $5 < 100$. χ^2 for the deviation from the distribution 7,5 : 7,5, theoretically expected, is 1,667 ($0{,}2 > P > 0{,}1$). Thus, the seed-setting of the hybrids is not significantly better than that of the pure lines, but the tendency is the same as in *purpureum*.

In the *Lamium* species studied the self-fertilization generally occurs in such a way that the anthers rub against the stigma by the stretching movements of the filaments when the flower opens. If this mechanism should fail, the anthers nevertheless in most cases will attain such a position on both sides of the stigma and so close to it as to bring about a self-pollination easily. It occasionally happens, however, that the self-fertilization will fail. The cause of the better seed-setting in the hybrids than in the pure lines might then primarily be suggested to be a somewhat better ability of self-fertilization, attained by a still better co-operation of the anthers and the stigma. It seems difficult to imagine that the reason for the failure of a full seed-setting could to any essential degree be anything but difficulties of some kind concerning the pollination, whether with reference to pure lines or to their hybrids, nor, *e. g.*, that the percentage of egg-cells or E.S's capable of functioning should be higher in the latter category than in the former.

The suggestion of a safer self-pollination in the hybrids might be supported by the fact that the only thorough investigation of self-fertilizing ability as yet made in *Lamium* has shown the hybrid plants to have a safer functioning mechanism of self-pollination than the only one of its parent lines investigated (BERNSTRÖM, unpublished). This investigation was made on *L. amplexicaule*, the intraspecific hybrids of which are characterized by vegetative

heterosis, as are the hybrids of *purpureum*. If the supposition about the cause of the higher seed-setting in the hybrids be correct, it may be put in the very category of the heterotic phenomena, the more regular self-fertilization being caused by a better development of the floral organs of the hybrids analogous to the predominantly better vegetative development of the hybrids as compared to their parent lines. However, as was pointed out above, it has not been possible to trace in *hybridum* any general vegetative heterosis of the kind suggested for *purpureum*.

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A. HAGBERG: Genotype and phenotype in alkaloid content in lupines.

In 1928 and 1929 v. SENGBUSCH (1942) made a selection of individual plants with a very low content of alkaloids in some annual species of *Lupinus*; his selections within *Lupinus luteus* (yellow lupine) and *Lupinus angustifolius* (blue lupine) have been widely grown in Germany, and also in Sweden under the name of »sweet lupine». It has proved very difficult to maintain a sweet stock; the percentage of »bitter» plants with a comparatively high content of alkaloids tends to increase, which can partly be explained by the fact that »bitter» plants seem to be more vigorous in the vegetative stage as well as in the production of seeds (v. SENGBUSCH, *l. c.*).

When grown on a large scale in Sweden, the sweet lupine has gradually increased its content of alkaloids, *i. e.* its bitterness. When an anlysis is made on seeds, only a few plants give the same precipitation with iodine as do typically »bitter» plants, but a considerable part of the seeds (10 % or more) give a precipitation, enough to classify the mother plant as »bitter». The occurrence of »semi-bitter» plants is, so far, not definitely genetically explained, but it seems probable that »modifiers» may increase the alkaloid-content of sweet lupines, and that the frequency of semi-bitters is increased by selection, and by recombination of such modifiers after cross-pollination (see below).

The breeder must know the genotype of his material before flowering time; during flowering the »bitter» types spread their pollen widely. Special studies made in the South of Sweden have given 25 % as an average of spontaneous cross-pollination in yellow lupines (HAGBERG, unpublished). The breeder, thus, must analyse the young plant. He then meets the problem of how soon the genotype is expressed by phenotypical characters.

As regards seeds, the relation between genotype and phenotype is well known (v. SENGBUSCH, *l. c.*) and may be summarized as follows (see next page, at the top): The content of alkaloids in the seed is dependent upon the genotype of the mother plant.

The main problem to the breeder, a problem which also offers some aspects of theoretical interest, is: At what stage in the ontogeny does the ge-

Maternal plant	Paternal plant	Seed character Phenotypical	Genotypical
sweet	bitter (homozygous)	sweet	bitter
sweet (gene *a*)	sweet (gene *b*)	sweet	bitter
bitter (homozygous)	sweet	bitter	bitter
bitter (heterozygous)	sweet	bitter	½ bitter ½ sweet

netical constitution break through the influence from the mother plant? — As material for the study here presented, crosses have been used within *Lupinus angustifolius*, the »sweet» variety Borre being used on one hand, »bitter» blue on the other.

The methods used to determine alkaloids are recommended by V. SENGBUSCH (*l. c.*), and depend upon the precipitation of alkaloid by iodine. The results are summarized in Tables 1 and 2.

TABLE 1. *The alkaloid reaction in 9 F_1 plants from the cross sweet × bitter.*

Field No.	The alkaloid reaction of seed, sowing date 5/4	leaves 13/4	19/4	29/4	4/5	12/5	27/5	2/6	21/6	11/7	26/7	ripe seed
213 — 1	0	0	—	0	0	+?	—	0	+?	+	+	K
— 4	0	0	0	0	0	0	+?	+?	+?	+	+	K
— 5	0	0	—	0	0	+?	0	0	0 ?	+	+	K
215 — 1	0	0	—	0	0	0	0	0 ?	+?	0	0	K
— 3	0	0	0	0	0	+	+	+	+	0	0	5
— 4	0	0	0	0	0	+	+	+	+	+	0	K
— 5	0	0	0	0	0	0	+	+	+	+	+	K
216 — 1	0	0	0	0	0	+	+	+	+	+	0	K
217 — 2	0	0	0	0	0	0	+?	+?	0 ?	+?	+?	K

Table 1 shows the results of nine F_1 plants of the cross sweet × bitter. (For analyses of the vegetative parts + means obvious reaction = bitter, +.? a somewhat dubious reaction, 0? a very weak reaction, 0 no reaction whatever = sweet, — no test made. Seeds are classified as 0 = no reaction, 1—5, different stages of »intermediate» bitterness, and K = reaction equal to pure bitter lupine.) The material shows that seeds from a »sweet» mother plant are »sweet», and that the leaves of the offspring start to produce or contain alkaloids about 5—6 weeks after sowing. Though the analysis of *leaves* is at no time fully reliable, the analysis of the *seeds* of the F_1 plants seems to be nearly completely reliable. Weather conditions, such as temperature, sunshine, drought, seem to have a considerable influence on the development of alkaloid in semi-bitter and bitter plants.

Forty seeds in the F_2 from the cross »sweet» × »bitter» *Lupinus angustifolius* gave rise to fullgrown plants. Among these 12 gave none or an extremely faint precipitation with iodine so that they were classified as »sweet», 28 plants were classified as »bitter». The segregation into 28 bitter : 12 sweet fits the 3 : 1 ratio.

The ontogeny as regards alkaloid-content of the 12 sweet plants, and of five others with a precipitation of their seeds in classes 4—5, is given in Table 2. (The other 23 bitter plants gave + in every analysis.) The seeds are all bitter when borne on a bitter (heterozygous) plant. The bitterness disappears gradually, but even at flowering time, around 23rd of June, the genotype of the plant has not quite overcome the influence, »inheritance», of the mother-plant.

TABLE 2. *The alkaloid reaction in some F_2 plants from the cross sweet×bitter.*

Field No.	The alkaloid reaction of										
	seed, sowing date 5/4	leaves									ripe seed
		13/4	19/4	29/4	4/5	12/5	27/5	2/6	21/6	11/7	26/7	
203 — 5	+	+	+	0	—	0	0	+	0	+	+	4
— 6	+	0	+	+	—	+	+	+	+?	+	+	1
— 17	+	+	+	+	+	+	0	+	0	0	0	0
— 28	+	+	+	+	+	+	+?	+	+	+	+	1
— 29	+	+	+	+	+	0	+	0	0	0	0	0
— 216	+	+	+	+	+?	0	0 ?	0 ?	0	0	0	0
— 220	+	+	+	+	+	+	+	0	0	0	0	0
— 227	+	+	+	+	+	+?	+	0	0	0	0	1
— 228	+	+	+	0	0	+	+	0 ?	0	0	0	4
— 229	+	+	+	0	0	+	+	0	0	0	0	0
— 230	+	+	+	+	0 ?	+?	+	0	0	0	0	0
— 233	+	+	+	+	0	0	+	+	+?	+	+	5
— 234	+	+	+	+	0	+	+	0	0 ?	0	0	4
— 249	+	+	+	—	—	+	—	+	0	0	0	0
— 250	+	+	+?	—	—	+	—	0	0	0	0	1
— 263	+	+	—	+	+	+	+	+	+	+	+	4
— 281	+	+	—	+	—	+?	+?	+?	0 ?	0	0	0

There is no full correlation between alkaloid content of leaves and that of the ripe seed, as is shown by v. SENGBUSCH (*l. c.*). The methods used for the determination of alkaloids in leaves (the results may be different if other parts of the green plant are used) are evidently not fully reliable. One thing seems to be evident, however, *viz.* that the maternal influence is very important. Plants from seeds on a »sweet» mother do not show their genetical »bitterness» until rather late in their ontogeny. Genetically sweet plants, borne on bitter mothers, do not show their sweet tendency until rather late. — The problem needs further investigation, but the persistence of »maternal» influence seems to make it worth while to publish a brief report at present.

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AXEL NYGREN: A preliminary note on cytological and embryological studies in arctic *Poae*.

During the last five years an investigation has been worked out in the *Poa* species growing in the Scandinavian mountains. The following species and forms have been studied: viviparous as well as non-viviparous *Poa arctica* R. BR, and *Poa pratensis* L. subsp. *alpigena* HIITONEN, viviparous *Poa alpina* L., non-viviparous *Poa laxa* HAENKE subsp. *flexuosa* (SM.) HYLANDER, and, in addition, the viviparous hybrids between *Poa alpina* L. var. *vivipara* L. on the one hand and *Poa arctica* R. BR., *Poa laxa* HAENKE subsp. *flexuosa* (SM.) HYLANDER, and *Poa pratensis* L. subsp. *alpigena* HIITONEN on the other hand.

Viviparous *Poa alpina* forms pollen and may cross with other *Poa* species growing in its distribution area.

Poa laxa subsp. *flexuosa* constitutes in Scandinavia a sexual and autogamous species with $2n = 42$. It grows often solitary on the summits of the mountains but has, in spite of this, very good seed setting, a circumstance that depends upon the fact that the anthers dehisce before the florets open. The development of pollen is disturbed in different ways and giant grains are often formed. It is very probable that the other subspecies in *Poa laxa*, distinguished by NANNFELDT in 1935, behave in the same manner as Scandinavian *flexuosa*.

Poa arctica is very polymorphous in Scandinavia and has chromosome numbers running from $2n = 39$ to $2n = 92$. In 1940 NANNFELDT distinguished six subspecies, three of which occur in the Dovre district of Central Norway, *viz. depauperata, elongata*, and *stricta*. The two first mentioned ones have fairly high chromosome numbers, varying between $2n = 68$ and $2n = 79$. Both subspecies form aposporous E.S's and are characterized by a selected mechanism of an early first division of the egg-cell, which makes every cross with other *Poa* species impossible with these *arctica* forms as mother plants — *Stricta* is viviparous but has a sexual development of the embryo-sac. Besides this, *stricta* has the lowest chromosome number known in *arctica, viz.* $2n = 39$, and for these reasons it is to be regarded as the last remnant of a very old sexual population. The low number has been preserved because of the fact that the plant is viviparous.

In Northern Scandinavia NANNFELDT has distinguished the three subspecies *caespitans, microglumis*, and *tromsensis* out of the *arctica* population.

Tromsensis has not been investigated but there is all reasons to suspect that it behaves just as *depauperata* and *elongata*. — *Microglumis* has chromosome numbers varying between $2n = 68$ and $2n = 82$. The writer presumes that this subspecies may be formed and recombinated now and then by suitable crosses between different *arctica* strains. — *Caespitans* has 56 chromosomes somatically and does not form any pollen. In its main locality in Scandinavia there are, however, other forms very similar to *caespitans* with the same chromosome number but having pollen. The subspecies develops sexually as well as aposporously, the egg-cells of the E. S's may rest or divide at an early moment, and thus *caespitans* has chances to form hybrids and, in addition, to propagate asexually. The endosperm has the following chromosome numbers: ca. 84 ($3n$), 112 ($4n$), and ca. 140 ($5n$). The number 112

is of interest because of the fact that in this case the central nucleus has *not* been fertilized, in spite of the agamospermous *Poa* species being pseudogamous.

Viviparous as well as non-viviparous *alpigena* behave just as *Poa pratensis* L. subsp. *eupratensis* HIITONEN. The moment when the egg-cell divides varies in different strains. It is important that viviparous *alpigena* develops pollen, because for that reason it may cross with other *Poa* species.

The hybrid between *Poa alpina* var. *vivipara* and *Poa arctica,* which has been detected quite recently, as well as the hybrid between *P. a. vivipara* and *P. laxa flexuosa,* which has been known long ago, are both viviparous and sexual as to the development of the E.S. They do not form seeds and they have very poor pollen. The hybrid between *P. alpina vivipara* and *P. pratensis alpigena,* on the other hand, forms pollen and may give seeds in a sexual or an aposporous way in spite of its being viviparous. Most chromosome numbers between $2n = 47$ and $2n = 80$ occur in this hybrid, which bridges the gap between viviparous *Poa alpina* and viviparous *Poa pratensis alpigena.*

The innumerable number of viviparous and non-viviparous arctic *Poa* forms occurring in the Scandinavian mountains as well as in the Arctic have originated after crosses between apomictic, facultatively sexual, and sexual types, in the later generations of which new sexual and apomictic forms have arisen. Some of these forms propagate by vegetative means exclusively, while other ones may also form new types by crossing. In this way the number of forms increases every year, provided that the outer conditions do not change.

Literature cited.

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